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FLOUR BLEACHING WITH CHLORINE DIOXIDE[1]

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The use of chlorine dioxide for flour bleaching was discussed by Staudt (1928) in a British patent in which improvements in flour baking characteristics and color removal similar to that achieved by the use of nitrogen trichloride were reported. A treatment of one or two grams of chlorine dioxide per 100 kilograms of flour was recommended.

Another patent was granted to Becher (1933) in which claims were made covering the use of combination bleaching treatments involving chlorine dioxide with nitrogen oxides, nitrosyl chloride, and organic peroxides. The authors have encountered nothing in the scientific literature describing the use of chlorine dioxide as a flour bleaching agent.

Despite the good bleaching results possible with chlorine dioxide, little interest has been shown in the reagent by flour millers, perhaps because no cheap and reliable method has been available for producing the gas. However, by 1935 the Mathieson Alkali Works, Inc., developed means for producing chlorine dioxide economically from a technical grade of sodium chlorite which they manufacture. Chlorine dioxide can conveniently and economically be produced from technical sodium chlorite either by the electrolytic process of Logan (1939) or by the solution process of Cunningham and Losch (1936).

Experimental

In the present studies on the treatment of flour with chlorine dioxide, the electrolytic method of Logan (1939) was used for generating the gas. This method consists essentially in the electrolysis of a solution containing about 144 g per liter of $NaClO_2$ and 140 g per liter of NaCl

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in a cell in which the anode and cathode compartments are separated by a porous diaphragm. Suitable materials for the electrodes are graphite, nickel, and copper.

The solution is fed continuously into the anode compartment and after electrolysis is disposed of through an overflow in the cathode compartment. Chlorine dioxide is formed at the anode and is stripped from the solution by a stream of air which keeps the partial pressure of the chlorine dioxide below explosive levels. The partial pressure of the chlorine dioxide in the chlorine dioxide-air mixture may be further reduced by dilution with more air after leaving the cell. The chlorine dioxide-air mixture is then led into an agitator suitable for flour bleaching. Chlorine dioxide obtained by this process is substantially free from chlorine. Control of the chlorine dioxide output is effected through control of the current. Approximately an hour must be allowed for the cell to reach equilibrium, during which time the solution feed, current, and air flow for stripping off the chlorine dioxide are carefully controlled and maintained at constant rates. Each cell must be calibrated for chlorine dioxide output with different values of current, solution feed, and air flow. For batch bleaching purposes for the treatment of a few pounds of flour, it is convenient to calibrate a cell at one current value, one air flow (for stripping), and one feed flow. The dosages on the flour may then be regulated by using the constant output of the cell for varying lengths of time.

Chlorine dioxide reacts practically instantaneously with flour as do the other gaseous reagents in common use, such as chlorine, nitrogen trichloride, and nitrogen peroxide.

It was found in general that less chlorine dioxide is needed to bring about optimum bread-baking characteristics in flour than is the case with nitrogen trichloride. From 0.3 to 1.5 g of chlorine dioxide per barrel of flour is usually sufficient to achieve the same maturing action that can be secured with about twice as much nitrogen trichloride. It is essential, therefore, to control accurately the amount of chlorine dioxide applied to flour in order to avoid overtreatment.

Chlorine dioxide is very efficient as a color-removing or bleaching agent for flour. Used at the maximum rate that is compatible with optimum baking characteristics, it will usually oxidize more carotinoid pigments than the optimum amount of nitrogen trichloride applied to the same flour. In fact, it will often bleach flour satisfactorily without the aid of any other bleaching agent, whereas compositions containing benzoyl peroxide are usually used to supplement bleaching treatments with nitrogen trichloride. Figure 1 shows typical examples of the relationship between the amount of chlorine dioxide applied and residual coloring matter in a patent and a clear flour.

It is evident from the curves in Figure 1 that the greatest color removal with chlorine dioxide occurs with the first increments of the gas applied to a patent flour. However, in the case of a first clear flour, a substantial amount of chlorine dioxide must be applied before much color removal results. At this writing, commercially bleached patent flours usually contain between 1.3 and 0.6 ppm of carotinoid pigments (expressed as "carotene") determined by the naphtha-alcohol extraction procedure. Figure 1 shows that these "carotene" levels are reached when 0.6 to 1.4 g of chlorine dioxide per barrel of flour is applied to the

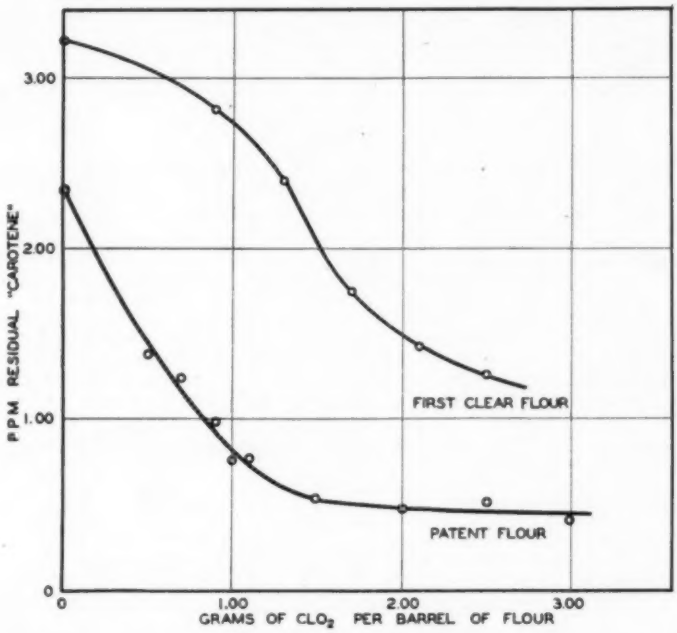

Fig. 1. Carotinoid pigment content of chlorine dioxide bleached flours—naphtha-alcohol extraction basis.

patent flour in question. Experiments with many patent and clear flours have demonstrated that the shape of the curves shown in Figure 1 is characteristic for each type of flour.

With most reagents used for flour bleaching, there is a correlation between flour slick color and bread crumb color. In the case of chlorine dioxide, however, this correlation is not found consistently. There appears to be some tendency towards dullness in the slick score of flours bleached with this reagent, but no corresponding dullness is found in the bread crumb color. In fact some very white crumb colors with no trace of dullness have been found in bread made from flours which produced

quite dull slicks, showing that in the case of this reagent, the slick does not have its usual significance, bread crumb color being the best and most significant criterion. Of course when flour is overbleached with chlorine dioxide, the crumb color will become dull just as in the case of overbleaching with any other reagent.

Chlorine dioxide exerts a maturing action on flour similar to that produced by nitrogen trichloride. Frequently the grain and loaf volume of bread are improved by chlorine dioxide treatment. While similar improvements are achieved in most cases by nitrogen trichloride, instances have been encountered in which either one or the other reagent is the more effective. Chlorine dioxide affects the dough-handling properties of some flours, producing a dryer, easier handling dough. Overbleaching with chlorine dioxide produces a "bucky" type of dough.

TABLE I

CHLORINE DIOXIDE BLEACHED PATENT FLOUR—COLOR AND BAKING CHARACTERISTICS [1]

Bleaching treatment per barrel of flour	Carotinoid pigments [2]	Slick score	Absorption	Crumb		Loaf volume
				Color	Grain	
	ppm					*ml*
Unbleached	2.92	Yellow	64	Yellow	10	2740
Mill bleached flour	1.29	10	64	10	10	2795
0.5 g ClO_2	1.71	9 creamy	65	8 creamy bright	10	2830
0.8 g ClO_2	1.18	10 slightly dull	66	11 bright creamy white	10	2815
1.5 g ClO_2	0.75	8 dull	66	13 white	10	2685

All other baking characteristics were the same for the mill bleached and chlorine dioxide bleached flours.

[1] Commercial formula, straight-dough procedure. One-pound loaves. Three hours' fermentation.
[2] Expressed as carotene. Naphtha-alcohol extract.

Some of the color and baking characteristics of chlorine dioxide bleached flours are shown by way of example in Tables I and II for patent and first clear flours respectively. In Table I it is seen that the optimum slick score is achieved at a dosage of 0.8 g of chlorine dioxide per barrel of flour, a greater treatment causing a decrease in the slick score. However, the crumb color of the bread from patent flour becomes whiter as the dosage of chlorine dioxide is increased to 1.5 g per barrel of flour. The color removal becomes greater, of course, as the chlorine dioxide dosage is increased. It can be seen in Table I that the optimum treatment from the standpoint of loaf volume is less than 1.5 g per barrel. While more closely graduated dosages would indicate

the optimum treatment more accurately, the data in the tables illustrate typical trends.

Table II illustrates the color and baking characteristics of a first clear flour treated with varying dosages of chlorine dioxide. In this case the slick score increases until a treatment with 2.1 g of chlorine dioxide per barrel of flour is reached and then at 2.5 g per barrel it falls off rapidly. Just as with the patent flour, the crumb color score increases as the chlorine dioxide treatment of the flour is increased. In this case no appreciable falling off in volume occurs with chlorine dioxide treatments up to 2.5 g per barrel. It can be seen that the grain is improved by both nitrogen trichloride and chlorine dioxide treatments, but only 1.7 g of chlorine dioxide is needed to bring about as much improvement in the grain as 5 g of nitrogen trichloride per barrel of flour; further, 1.7 g of

TABLE II

CHLORINE DIOXIDE BLEACHED FIRST CLEAR FLOUR—COLOR AND BAKING CHARACTERISTICS [1]

Bleaching treatment per barrel of flour	Carotinoid pigments [2]	Slick score	Crumb		Loaf volume
			Color	Grain	
	ppm				*ml*
Unbleached	2.64	Yellow	Yellow	7 spherical harsh	585
5 g NCl_3 (*control*)	1.50	10	10	10	630
0.9 g ClO_2	2.32	7 creamy	6C	8 spherical	610
1.3 g ClO_2	1.96	9 creamy	9C	9 spherical	595
1.7 g ClO_2	1.34	11	11	10	620
2.1 g ClO_2	1.05	12	11+	10	620
2.5 g ClO_2	0.93	7 dull	12	10	615

All other baking characteristics were the same for both the chlorine dioxide and nitrogen trichloride treated flours and the unbleached flour.

[1] Basic A.A.C.C. formula plus 0.25% Arkady.
[2] Expressed as carotene. Naphtha-alcohol extract.

chlorine dioxide in this instance has given better color removal than 5 g of nitrogen trichloride, as evidenced by the "carotene" and crumb color figures given in Table II. The data illustrate the greater effectiveness of unit dosages of chlorine dioxide. Presumably chlorine dioxide is a more potent reagent than nitrogen trichloride for flour bleaching purposes.

Chlorine dioxide has very little effect on the pH of flour in the amounts in which it is used for bleaching. From this standpoint it is not a substitute for chlorine. The effect of chlorine dioxide on pH is usually less than that of a nitrogen trichloride and benzoyl peroxide combination

bleaching treatment, due in part, no doubt, to the fact that much less chlorine dioxide is needed to treat flour than nitrogen trichloride.

Chlorine dioxide alone has been found capable in some instances of substituting for commercial bleaching treatments such as nitrogen trichloride and benzoyl peroxide, nitrogen trichloride alone, benzoyl peroxide alone, or chlorine and benzoyl peroxide.

In order to preserve the keeping qualities of flours treated with chlorine dioxide, it has been found desirable to apply less than 2 g per barrel of this reagent. If sufficient color removal cannot be obtained with the application of 2 g or less of chlorine dioxide, then benzoyl peroxide may be used to achieve additional color removal.

Summary

Chlorine dioxide is an effective flour bleaching and maturing agent. Compared with other commercial bleaching agents, smaller dosages in terms of grams per barrel are required; hence its application requires accurate control. As anticipated, clear flours require heavier dosages than patent flours.

When chlorine dioxide is used in the amount needed to give optimum baking characteristics, it usually removes more color than the optimum quantity of nitrogen trichloride on the same flour. Grain and loaf volume usually reflect the maturing action of chlorine dioxide. Dough-handling properties are affected sometimes, a dryer feeling dough being obtained. The pH of flour is not affected by the quantity of chlorine dioxide used in flour bleaching. For optimum keeping quality, the dosage should be kept preferably below 2 g per barrel of flour.

Acknowledgment

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EFFECTS OF MOISTURE ON THE PHYSICAL AND OTHER PROPERTIES OF WHEAT

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Wheat, to be of best quality, as measured by test weight and color, should not receive any added moisture after it is dry-ripe. When wheat contains 30% to 35% moisture, it is mature enough to harvest with a binder without shriveling. Further physical changes in the wheat are due mostly to desiccation. When wheat is to be harvested with the combine, the moisture content should not be more than 14%, preferably less. If wheat is wetted by rain after it is dry-ripe more or less change occurs, the extent depending on the amount of rain and the prolongation of wet weather. Showers followed by sunshine are not as damaging as prolonged drizzles with much cloudy weather. However, even small showers followed by sunshine may affect color and test weight in such a manner as to influence the grain grading properties.

Since test weight is an important factor in grain grading, its being lowered by wetting after the wheat is dry-ripe causes large losses to the wheat growers. Fifty-one-pound test weight wheat, badly bleached because of prolonged rains after it was ready for the combine, has been observed, yet this wheat appeared plump, and the indications were that the flour yield would not be lowered in proportion to the decrease in test weight.

Previous Investigations

The percentage of yellowberry was not influenced in samples of wheat cut at various stages of maturity and then subjected to various conditions of wetting and drying (Swanson, 1936). It was indicated that yellowberry was caused by conditions existing prior to the cutting of the wheat. The test weight was lowered, the amount depending upon the number and duration of the wetting periods. Diastatic activity was influenced only when the amount of wetting was sufficient to stimulate the process of germination (Swanson, 1935).

Mangels and Sanderson (1925) found a high positive correlation in each crop year between average test weight and average flour yield. Bailey (1923) reported differences in flour yield of 2.5% from different lots of 60-pound wheat and 3.6% from 58-pound wheat.

Bracken and Bailey (1928) harvested and threshed wheat just as soon as the wheat was ripe and repeated at ten-day intervals, six cuttings

and threshings, covering 50 days. They found that three rains totalling 0.69 inch completely bleached the wheat and reduced the test weight four pounds per bushel, due to an increase in kernel volume and a decrease in density. Kernel sections were shown to be opaque. Subsequent rains did not produce further decrease in test weight.

Sharp (1927) found that "after the moisture content of the once air-dried wheat has been increased and the moisture again removed, the wheat does not regain its former density, the decrease depending on the amount of moisture taken up. When corneous kernels were increased in moisture content to between 18% and 25% and the moisture reduced again by drying, the resulting kernels were opaque and when cut were invariably starchy in appearance. The decrease in density is due to the formation of air spaces in the kernel after the removal of the water."

Whitcomb and Johnson (1930) threshed wheat at monthly intervals at Bozeman, Montana, from shocks exposed to the weather from August until the following March. The prevailing weather in the fall was dry and the winter was unusually cold. "Test weight per bushel showed a greater change as weathering progressed than did any other property of the wheat considered." For Marquis the maximum decrease in test weight was 5.2 pounds and for Kanred, 6 pounds. "The loss in test weight was due to decrease in specific gravity caused by the swelling of the kernels when they became wet and their failing to assume normal size when they dried out." Whitcomb and Johnson observed that the dark kernels decreased from 86% to 17% for Marquis, and from 72% to 34% for Kanred.

Relation of Flour Yield to Test Weight

Differences in reports on the correlation or lack of correlation between test weight and flour yield arise in part from a failure to distinguish between test weight as influenced by shape of kernel caused by weather conditions during ripening or by factors of inheritance, and test weight as influenced by moisture conditions after the wheat is dry-ripe. The percentage of endosperm in the kernel increases constantly during the process of development (Bailey, 1925). If the wheat is cut before endosperm development is completed or if the full development is prevented by very hot and dry weather, the percentage of endosperm will be decreased and as a consequence the flour yield will be lowered. The test weight of such wheat, because it is correlated with plumpness, will be a good criterion of the probable flour yield. That variety is also a factor is shown by the generally higher test weights of Blackhull and Chiefkan as compared with Kanred and Tenmarq. It has not been satisfactorily demonstrated that the flour yields of the two former varie-

ties are correspondingly higher. However, when the test weight is lowered because of kernel swelling and roughening of the bran coat due to wetting, no change in the proportion of endosperm and bran occurs, and the flour yield is not decreased in proportion to the lowered test weight.

The swelling of the kernels, due to wetting, disorganizes the more dense original structure and, hence, increases the internal air space. The scattering reflection of light by these irregularly distributed and variously shaped air capillaries causes the opaque or mealy appearance, similarly as the innumerable air bubbles give the white color to the Easter lily.

Outline of the Experiments

The object of the experiments reported in this paper was to determine the effects of wetting and drying wheat, both before and after threshing, on several factors used in evaluating quality. The wetting and drying before threshing had two phases: (1) wetting bundles from one to four times and then drying, one set in the sun and another in the shade; and (2) exposing wheat in the field in four shocks for about two months. The wetting and drying of the threshed grain was done in the laboratory as will be described later. The measurements for quality made on these samples were as follows:

1. Test weight was determined by the official method.
2. Color, texture and general appearance of the grain were obtained by two methods: (1) Envelope samples were submitted to Martin Shuler of the Kansas City office of the Grain Inspection Department for official grading. (2) A barley kernel cutter was used to cut 50 kernels into halves, exposing the cut sections for observation.
3. Milling tests were made to obtain flour for other measurements and to determine to what extent flour yield was influenced by changes in the test weight.
4. Pearling tests were made to determine hardness of the grain.
5. The diastatic activity was determined on all the samples treated in the straw and on a few of those treated in the grain. No effects in the latter were obtained.
6. Baking tests on selected samples were made to determine the effects of the various treatments on loaf volumes and bread textures.
7. The carotene content was determined on selected samples representing the extremes, but the amounts obtained were not correlated with the treatments.
8. Dough-mixer curves were made on a few representative samples.

Turkey wheat from a uniform field was used.[1] No rain fell during the 1940 harvest from the time the wheat was maturing until the latter part of July, except for a trace on June 23. Hence, wheat was obtained both in bundles and as threshed grain which had not been appreciably wetted during ripening or after desiccation in the field.

Treatment of unthreshed wheat.[2]—Enough wheat was cut on June 22 to furnish bundles for four large shocks and for wetting and drying in the shade and sun. The wheat was in a condition fit to cut with the binder, the straw was all yellow, and the grain was in the very hard dough stage.

One shock was covered with a water-proofed canvas securely anchored; another was capped with bundles securely tied so as not to blow off; a third shock had no cover or cap bundles; and the fourth was built like the third, but from this bundles were taken and threshed at various intervals as follows: July 1, July 24, July 29, August 13, and September 5. The first two of these threshings had had no rain except a trace on June 23. The rainfall during July and August was as follows:

July 27.....0.29 inch	Aug. 16.....0.60 inch
Aug. 8.....1.35 inches	Aug. 17.....0.40 inch
Aug. 13.....0.30 inch	Aug. 22.....0.06 inch
Aug. 14.....0.75 inch	Aug. 26.....0.72 inch
Aug. 15.....0.31 inch	Aug. 27.....0.38 inch

The wheat which was threshed on July 29 was wetted by one 0.29-inch rain; that threshed on August 12 received two rains amounting to 1.64 inches. Ten rains totaling 5.16 inches had wetted the shocks of the final threshing made September 5. At this threshing the uncovered shock and the shock capped with bundles were each divided into two portions, outside and inside bundles, but the canvas-covered shock was not divided. The remaining bundles from the fourth shock were threshed as the final sample.

The bundles for the wetting and drying in the sun and shade were divided into two groups, and separate bundles in each group were wetted according to the following scheme:

	Times Wetted	
Group 1:	0, 1, 2, 3, 4	Dried in the shade after each wetting.
Group 2:	0, 1, 2, 3, 4,	Dried in the sun after each wetting.

The bundles were wetted by immersing the heads in water for a definite number of minutes, but for each group there was one lot that received no wetting. The first soaking was for 30 minutes, the second for 20

[1] Acknowledgment is hereby made to the Department of Agronomy for assistance both in obtaining uniform wheat and operation in the field.
[2] The routine work in all the experiments, except baking, reported in this paper was performed by Willard Meinecke, student assistant. His painstaking attention to details is hereby acknowledged.

minutes, the third for 10 minutes, and the fourth for 5 minutes. The drying proceeded very slowly after the third and fourth soakings in the bundles dried in the shade and the straw turned dark. To hasten drying, the straw in each bundle was turned so that what was on the inside of the bundle came to be on the outside. No rain fell, but on some days the sky was overcast.

Each lot of the bundles dried in the sun were placed in the shade as soon as they were dry after the designated number of soakings. After the third wetting some sprouting was observed in some of the bundles dried in the shade. All these bundles were threshed July 8.

Treatment of the threshed wheat.—The wheat used for wetting as grain was cut July 1, and five bushels of grain threshed as soon as practicable. Since the last rain occurred on June 11, the wheat had not been wetted since it was ripe. The wheat was cleaned in the laboratory separator and then exposed in shallow layers so that the moisture content should become low and uniform. The moisture content of a composite sample was 10.3%, vacuum oven, and the test weight 61.3 pounds.

The general plan was to place 1800-gram portions in gallon bottles; add water to make the moisture percentages of the various portions to 12, 14, 16, 18, 20, 22, 24, 26, and 28%; shake well so as to distribute the water evenly; determine the test weights as soon as the water was absorbed; then expose in shallow paper boxes until dry; and then determine the test weight of the dried samples. The exposure to the dry laboratory air was continued for each sample until the weight was near 1800 grams. Tests showed that a few grams of variation had no significant influence on test weight. Wetting and drying were repeated from one to six times at the 2% moisture intervals from 12% to 28%, inclusive. Thus the wheats in the bottles of Lot 1 were wetted once, test weight taken in duplicate, then dried and test weight taken. This process was applied the same number of times as the lot numbers. Thus the wheat in Lot 2 was wetted twice and dried after each wetting; three times for Lot 3; four times for Lot 4; five times for Lot 5; and six times for Lot 6.

The dry, hot weather in July, 1940, favored the rate of drying after each wetting. As soon as the samples were dry, they were returned to the original bottles until the next treatment. A 1400-gram portion was taken from each of the dried samples for milling; an 8-ounce bottle for reserve; 75 grams for grading the grain; and the remainder was for use in determining hardness by means of a barley pearler, and also for estimating the counts of mealy, semivitreous, and vitreous kernels with the use of a barley kernel cutter.

Results of Treatments Before Threshing

The data obtained from the various treatments before threshing are given in Table I. The moisture content was low in the samples threshed before the rains started, but the percentages were higher in those from the shocks threshed the latter part of August and the first part of September. No rain had fallen between August 27 and September 5 when the last threshing was done. The wheat in the covered shock had 12.3% moisture. About 2% had been absorbed from the more humid atmosphere which prevailed during August. Atmospheric conditions also prevented the drying out of the wheat wetted by rains in the exposed shocks to the level that was obtained when the wheat was threshed during the very dry weather.

TABLE I

TREATMENTS: TEST WEIGHTS, MALTOSE VALUES, AND PERCENTAGES OF MOISTURE, FLOUR YIELD, AND FLOUR ASH ON SAMPLES TREATED BEFORE THRESHING

Treatment	Number wettings	Threshed	Moisture wheat	Test weight		Flour yield	Flour ash	Diastatic activity
				Cleaned	Scoured			
		date	%	*lbs.*	*lbs.*	%	%	*mg. maltose*
Bundles:								
Soaked and dried in sun	0	7/8	10.0	60.5	63.2	71.8	0.43	112
" " " " "	1	7/8	10.4	60.4	62.8	72.6	0.42	117
" " " " "	2	7/8	10.1	60.5	63.0	72.4	0.40	105
" " " " "	3	7/8	10.3	58.9	62.0	70.0	0.39	111
" " " " "	4	7/8	9.9	58.7	61.7	69.9	0.38	109
" " " " shade	0	7/8	10.1	60.2	62.9	72.8	0.40	140
" " " " "	1	7/8	10.4	60.1	63.0	72.6	0.40	139
" " " " "	2	7/8	10.3	58.1	61.2	69.4	0.40	156
" " " " "	3	7/8	10.3	58.3	61.3	68.4	0.38	212
" " " " "	4	7/8	10.2	57.8	61.3	68.2	0.38	267
Exposed shocks:	Rain, *in.*							
Not capped:								
Sample 1	0.00	7/1	10.0	60.3	62.9	72.6	0.46	135
" 2	0.00	7/24	10.9	60.1	63.1	73.6	0.46	122
" 3	0.29	7/29	10.9	59.1	62.3	73.6	0.42	115
" 4	1.64	8/15	11.0	56.7	60.2	71.9	0.42	160
" 5	5.16	9/5	13.0	55.8	59.9	70.6	0.42	335
Covered	5.16	9/5	12.3	60.4	63.0	70.9	0.43	233
Capped—outside bundles	5.16	9/5	12.5	57.3	61.2	69.1	0.41	264
Capped—inside bundle	5.16	9/5	11.7	58.3	61.9	69.2	0.41	203
Not capped—outside bundles	5.16	9/5	13.3	55.1	59.6	69.2	0.42	330
Not capped—inside bundles	5.16	9/5	13.3	58.2	62.1	70.3	0.40	258

Effect of test weight on flour yield.—The various wettings as well as the exposure to the rains in the shocks decreased the test weight. The least change was in the samples dried in the sun, in the wheat from the covered shock, and from the two threshings from the shock before the rains came. The samples wetted several times and dried in the shade and the samples taken from the outside of the shocks decreased the most in test weight. On some of these bundles there was evidence of sprout-

ing. The larger test weights obtained after scouring indicate that the decrease in test weights of the wetted as well as the weathered samples was in part due to the roughened condition of the bran coat. Scouring made the kernels more smooth and allowed closer packing and hence greater test weight.

The flour yields showed a trend toward a decrease as the test weight became less in the samples wetted and dried in the sun or in the shade. However, since the ash percentages also show a trend toward a decrease, it is a question whether the lower flour yield may not have been due to a lower extraction. The samples obtained from the exposed shocks showed little or no correlation between test weight and flour yield as well as between ash content and flour yield. Neither did the various treatments have any consistent effect on the ash percentages.

Diastatic activity.—There was no correlation between diastatic activity and treatments in the samples wetted and dried in the sun or in the samples from the shocks threshed before the rains came. There was a considerable increase in diastatic activity in the samples wetted and dried in the shade, especially those wetted three or four times. The greatest diastatic activity was found in the samples taken from the exposed portions of the shocks in which there was evidence of sprouted kernels. Diastatic activity is greatly increased by conditions of germination (Swanson, 1936). The fact that the sample from the covered shock also had a high diastatic activity indicates that moist weather conditions which prevailed through August had an effect in increasing the diastatic activity. Since this was evident in only one sample, effect of high humidity on diastatic activity needs further investigation.

Results of grain grading.—The official grading of these samples indicated the numerical grade, their subclass, and the percentages of vitreous and damaged kernels as shown in Table II. The test weight (Table I) was apparently the principal factor in determining the grade in all but two samples, which were given sample grade. These two were from the bundles most exposed in the shocks and both of these had large percentages of total damage. The percentages of vitreous kernels show a distinct trend toward a decrease corresponding with the amount of wetting and exposure. The trend toward decrease in vitreousness also correlated with the increase in total damage.

Texture and hardness tests.—A barley kernel cutter was used to examine the cross sections of wheat kernels. The cutter has 50 single-grain pockets which hold the wheat kernels while they are cut into halves by a moving steel cutting blade. After the instrument is opened the cut half sections of the 50 kernels are exposed so that they may be examined. The average results from two counts of mealy, semivitreous, and vit-

TABLE II

GRADING OF WHEAT SAMPLES TREATED BEFORE THRESHING

Treatment	Number wettings	Test weight cleaned [1]	Grade	Vitreous	Total damage
		lbs.		%	%
Bundles:					
Soaked and dried in sun	0	60.5	1 DHW	84	0
" " " " "	1	60.4	1 DHW	83	0
" " " " "	2	60.5	1 DHW	72	0
" " " " "	3	58.9	2 DHW	75	0.4
" " " " "	4	58.7	2 HW	63	0
" " " " shade	0	60.2	1 HW	68	0
" " " " "	1	60.1	1 HW	73	0
" " " " "	2	58.1	2 HW	28	3.0
" " " " "	3	58.3	2 DHW	77	3.2
" " " " "	4	58.7	3 HW	64	2.2
Exposed shocks:					
Not capped	Rain, *in.*				
Sample 1	0.00	60.3	1 HW	70	0
" 2	0.00	60.1	1 DHW	87	0
" 3	0.29	59.1	2 DHW	75	0
" 4	1.64	56.7	3 HW	60	10.0
" 5	5.16	55.8	5 SGHW	28	16.4
Covered	5.16	60.4	1 HW	72	0
Capped—outside bundles	5.16	57.3	5 HW	33	11.8
Capped—inside bundles	5.16	58.3	2 HW	58	0.4
Not capped—outside bundles	5.16	55.1	5 SGHW	40	20.0
Not capped—inside bundles	5.16	58.2	3 HW	63	6.8

[1] These test weights are repeated from the data in Table I.

reous kernels from the wheat samples treated before threshing are given in Table III.

Taylor, Bayles, and Fifield (1939) have developed a useful test for estimating the hardness of wheat. In our laboratory a machine similar to the barley pearlers used in grain inspection was employed. The samples before and after pearling were weighed on a balance sensitive to 0.01 g. and the losses computed in percentages.

The pearling losses for the samples dried in the shade were larger than for the samples dried in the sun, with one exception. The lowest losses for the shock samples were obtained from the covered shock and from the one sample threshed before the rains started. The largest losses were thus obtained from the samples exposed the most, demonstrating that exposure to moisture caused softening of the kernels.

The wetting and drying of the wheat in the sun and shade did not have as great an effect as when exposed to rain in the shocks. The wetting period was apparently not long enough to soak the kernels, and it was mostly the outside surface that was affected. Roughening of the

TABLE III

INTERNAL TEXTURE OF THE WHEAT GRAIN AND LOSS IN PEARLING

Treatment	Number wettings	Mealy	Semi-vitreous	Vitreous	Loss in pearling
		%	%	%	%
Check	—	4	2	94	—
Bundles:					
Soaked and dried in sun	0	4	2	94	32.4
" " " " "	1	4	4	92	33.4
" " " " "	2	2	4	94	32.9
" " " " "	3	10	8	82	36.2
" " " " "	4	10	22	68	34.9
" " " " shade	0	10	12	78	34.7
" " " " "	1	8	2	90	33.9
" " " " "	2	4	4	92	34.5
" " " " "	3	2	6	92	31.9
" " " " "	4	4	10	86	38.5
Exposed shocks:					
Not capped:	Rain, *in.*				
Sample 1	0.00	10	6	82	37.6
" 2	0.00	2	10	84	34.8
" 3	0.29	4	10	86	39.5
" 4	1.64	30	20	50	40.3
" 5	5.16	40	24	30	41.1
Covered	5.16	8	8	84	33.6
Capped—outside bundles	5.16	58	24	18	38.0
Capped—inside bundles	5.16	26	22	52	36.6
Not capped—outside bundles	5.16	42	22	36	40.4
Not capped—inside bundles	5.16	34	18	48	36.4

bran surface lowered the test weights. Exposure in the shocks showed the greater effect of the rain, and the wheat from the more exposed bundles from the outside of the shocks had a much larger increase in the mealy kernels (or a decrease in the vitreous kernels) than wheat from the inside bundles which were more protected. Capping the shocks with bundles was not very effective in preventing the grain from becoming wet as the results from the capped and the uncapped shocks were not widely different. Covering with canvas, which entirely prevented wetting, was highly effective in preventing the development of the mealy condition. In the uncapped shock from which samples were periodically threshed, there was no increase in the mealy kernels before the rains came or with the 0.29-inch rain. The rains following produced a marked increase in the number of mealy kernels.

Results of Treatments After Threshing

Effect of amount of wetting on test weight.—The test weights on the wet, the dry, and the scoured samples that were wetted and dried after

threshing are given in Table IV. The largest decrease in test weight in proportion to the amount of water added took place in increasing the moisture from the original moisture of about 10.3% to 12% or 14%. Wetting beyond 14% decreased the test weights proportionately much less, and the decreases were still smaller in wetting above 20% and 22% moisture. It appears that at 20% and 22% moisture, nearly the maximum amount of swelling had taken place, since the changes produced beyond this were comparatively small.

Effects of number of wettings on test weight.—A single wetting decreased the test weight proportionately more than the subsequent wettings. But while the decreases in test weights for the first wetting were larger than for each of the subsequent wettings, the trend toward the decrease persisted up to the sixth or last wetting, especially for the dried and scoured samples. This can be seen by noting the total decreases (bottom row, Table IV) which were obtained by subtracting the test weights obtained from the samples wetted to 28% from the check figures. While the largest amount of kernel swelling occurred with the first wettings, the subsequent wettings produced additional amounts of swelling.

Causes of decrease in test weight.—The fact that the decrease in test weight persisted after the wetted samples had been dried to the original moisture content shows that after the structural arrangements of the interior of the wheat kernels have been disturbed by addition of water, the original compactness cannot be restored by drying to the original moisture content. The decrease in test weight of wheat wetted one or more times is due partly to roughening of the bran coat and partly to increase in the volume of the wheat kernels and not to any loss of material. An examination of cross sections of kernels from samples wetted the most showed that the vitreous condition present in the original wheat had been replaced by a chalky or mealy texture. When the endosperm contracts after swelling by water the starch cells and other substances remain in a more or less disarranged condition, leaving numerous air spaces throughout the endosperm. The reflection of light from a mealy endosperm is similar to that from snow, and the reflection of light from a vitreous kernel similar to that from ice.

The influence of the condition of the bran coat on the test weights is shown by the larger test weights of the scoured samples. These larger test weights were due to closer packing in the test kettle because of smoother kernel surface. The trends toward decreases in test weights in the scoured samples as a result of wetting and drying are similar to trends in the unscoured dried samples. That is, the decreases are proportionately greater for the smaller amounts of wetting and also for the one wetting as compared with the following wettings. This is similar

to the results obtained by Bracken and Bailey (1928) in which it was shown that the first rains produced the greatest decreases in test weight.

TABLE IV

TEST WEIGHTS AS AFFECTED BY WETTING AND DRYING

Moisture to which wetted	Times wetted					
	1	2	3	4	5	6
			WET			
%						
Check	61.3	61.3	61.3	61.3	61.3	61.3
12	59.6	59.3	59.2	59.0	59.2	58.6
14	58.1	57.9	57.6	57.6	57.8	57.1
16	57.3	56.6	56.4	56.4	56.0	55.5
18	55.8	55.1	54.3	54.2	54.1	54.7
20	54.0	53.3	52.9	52.6	53.2	52.1
22	51.9	51.6	51.1	50.8	51.8	51.5
24	50.6	50.6	50.0	50.0	50.0	50.5
26	49.8	49.8	49.0	48.9	49.0	48.9
28	49.7	49.2	48.5	48.7	48.8	48.9
Total decrease	11.6	12.1	12.8	12.6	12.5	12.4
			DRY			
%						
Check	61.3	61.3	61.3	61.3	61.3	61.3
12	60.1	59.5	59.3	59.2	59.3	59.0
14	58.5	58.5	58.4	58.3	58.1	57.7
16	58.1	57.5	57.8	57.6	57.1	57.1
18	57.7	57.1	57.1	56.9	56.4	56.7
20	57.3	56.5	56.9	56.8	55.8	55.7
22	57.1	56.5	56.4	56.3	55.8	55.6
24	56.8	56.3	56.1	56.1	55.5	55.5
26	56.8	57.0	56.0	55.8	55.4	55.5
28	56.5	56.1	55.9	55.6	55.3	54.7
Total decrease	4.8	5.2	5.4	5.7	6.0	6.6
			SCOURED			
%						
Check	64.3	64.0	63.5	63.4	63.5	63.5
12	63.3	63.0	62.8	62.3	62.6	62.0
14	62.2	62.1	61.9	62.2	61.7	61.0
16	62.0	61.2	61.7	61.1	61.0	60.3
18	61.6	61.1	61.1	60.4	60.3	59.7
20	61.5	60.6	60.5	60.2	59.8	59.5
22	61.4	60.5	60.4	59.9	59.6	59.1
24	61.1	60.4	60.3	59.7	59.6	59.0
26	61.1	61.0	60.2	59.6	59.5	59.0
28	60.8	60.2	60.0	59.4	59.4	58.2
Total decrease	3.5	3.8	3.5	4.0	4.1	5.3

Milling Results

The milling was done on the Buhler mill with a constant roll and feed setting. The proper mill settings were determined by making a number of millings on check samples. Fourteen hundred grams of each sample were milled. The amount of tempering water was calculated on the basis of the moisture content of the samples, which was found to be near 10%, varying ¼% above or below. At first it was assumed that tempering to 16% moisture would be best for the checks as well as for those which had been wetted the lesser amounts, and that those which had been wetted to the larger amounts should be tempered to less than 16%. A few trials, however, showed that those which had been wetted to the larger amounts also milled best when tempered to 16%, and hence this was the amount used for nearly all the samples. The tempering was done in two stages. All but 14 cc. of water was added the evening before milling and the last 14 cc. was added about 15 minutes before starting to mill. Because the milling room is air conditioned, it was possible to hold the relative humidity near 70% and the temperature near 80°F.

TABLE V

BREAK FLOUR FROM WETTED WHEATS

Moisture to which wetted	Times wetted					
	1	2	3	4	5	6
%	%	%	%	%	%	%
Check	12.9	12.6	13.0	12.8	12.6	13.3
12	12.4	12.2	13.2	12.7	12.3	13.2
14	13.2	12.1	12.0	12.8	12.1	13.0
16	12.2	12.5	12.0	12.8	12.5	13.1
18	12.6	12.3	12.4	13.1	12.9	13.8
20	13.8	13.9	13.0	14.5	13.4	15.3
22	13.3	13.9	13.9	14.9	14.9	15.9
24	13.8	14.1	14.1	15.0	14.8	15.4
26	13.9	13.6	14.2	14.6	14.2	14.7
28	14.1	14.4	14.7	14.1	14.4	13.8

Percentages of break flour.—As a general rule, more break flour is obtained from soft than from hard wheats. To determine whether these samples had softened by the water treatments, the break flours were weighed separately, and their tabulated percentages are given in Table V. The figures show a small trend towards larger percentages of break flour with the increased amounts and number of wettings, which indicates some softening but not as much as might be expected.

The percentages of total flour.—The percentages of total flour were calculated by dividing the total weights of flour obtained by the 1400 g.

of wheat used, and the data from the various samples are given in Table VI. This method of calculation involves a small error since the tempering water contributes to the amount of flour. Part of the tempering water goes to the bran and shorts and part to the flour. There is also the so-called "invisible loss" which in most cases amounted to about half the weight of the tempering water. However, since results from all the samples were treated the same, the figures are comparable.

There is no definite trend towards increase or decrease in the percentages of flour obtained as a result of the amount or number of wettings. The total decreases in test weight (bottom row, Table IV), of the dry, unscoured wheat was 4.8, 5.2, 5.4, 5.7, 6.0, and 6.6 pounds respectively for the number of times wetted. The flour yields, computed on the weight basis, were just as large for the samples whose test weights had been decreased from 4.8 to 6.6 pounds as for the check samples (Table VI). The evidence shows that the flour yield of wheat was not affected by the number of times the wheat had been subjected to wetting. This was due to the fact that the various wettings had not caused any loss in weight but only increased the space occupied by the

TABLE VI

TOTAL FLOUR FROM WETTED WHEATS

Moisture to which wetted	Times wetted					
	1	2	3	4	5	6
%	%	%	%	%	%	%
Check	73.9	73.0	74.6	74.7	75.3	74.6
12	73.2	73.2	76.3	75.0	73.8	74.6
14	74.0	74.5	73.0	74.8	74.0	74.6
16	73.0	72.2	73.0	74.3	74.0	72.6
18	72.0	72.6	72.2	73.0	73.6	74.3
20	76.1	75.7	73.0	75.2	72.5	74.3
22	75.0	73.7	73.1	74.8	76.1	76.0
24	73.5	73.0	74.9	75.4	75.0	76.2
26	75.0	73.6	74.0	75.4	75.3	75.2
28	73.6	73.8	75.0	75.2	76.3	75.4

kernels. A given volume of kernels will weigh less after wetting, but a given weight of kernels had the same proportion of endosperm from which the flour is made as before the decrease in test weight by wetting.

Ash content.—Flour yields must be considered in connection with their ash contents. Since the setting of the rolls will influence yield, this will also affect the ash percentage. But as has been mentioned, these samples were all milled with a constant roll and feed setting. Percentages of flour ash (Table VII) apparently do not show any definite or significant trends. Hence, the lack of correlation of the test weights

TABLE VII

ASH OF FLOURS FROM WETTED WHEATS

(15% moisture basis)

Moisture to which wetted	Times wetted					
	1	2	3	4	5	6
%	%	%	%	%	%	%
Check	0.43	—	—	—	—	—
12	0.43	0.39	0.46	0.41	0.41	0.43
14	0.42	0.41	0.44	0.41	0.41	0.43
16	0.40	0.43	0.44	0.42	0.40	0.43
18	0.42	0.43	0.40	0.42	0.41	0.44
20	0.42	0.41	0.43	0.40	0.41	0.42
22	0.41	0.40	0.42	0.43	0.44	0.42
24	0.43	0.41	0.42	0.43	0.41	0.43
26	0.40	0.43	0.41	0.44	0.43	0.43
28	0.43	0.45	0.41	0.43	0.44	0.41

(Table IV) with the flour yields (Table VI) are not due to nonuniform milling. Test weight gives the pounds of wheat in a given volume, while flour yield is computed in percent on the basis of weight, not volume.

Flour moisture.—The moisture percentages of the straight flours were fairly uniform as should be expected because the wheat was all tempered to the same moisture content and milled under uniform humidity and temperature conditions.

Tests for Hardness

A visual inspection of these samples showed that there was a definite trend towards decrease in the vitreousness of the samples or an increase in the mealiness, corresponding with the amounts and times of wetting. The pearling test, described in connection with the samples wetted before threshing, was made on all the samples of grain wetted to various percentages from one to six times. The results of these pearling tests are given in Table VIII.

TABLE VIII

LOSSES IN PEARLING INDICATING RELATIVE HARDNESS

Percentage to which wetted	Number of wettings					
	1	2	3	4	5	6
%	%	%	%	%	%	%
12	32.8	36.6	34.2	34.6	34.8	35.9
14	34.5	36.9	37.1	36.4	38.5	40.0
16	36.5	38.9	38.2	38.6	40.6	42.3
18	37.4	39.0	40.5	43.8	45.7	45.1
20	38.4	40.8	44.0	44.8	45.4	46.5
22	39.3	40.2	44.6	44.3	45.4	46.1
24	39.6	41.6	44.4	44.4	44.3	42.7
26	38.5	39.6	43.8	41.4	40.7	41.2
28	37.9	39.5	40.9	38.9	39.1	38.0

There was a definite trend in the increase of pearling losses from the 12% wetting up to the 20%–24% wettings, but beyond this there was a reversal in the trend. The reason for this is not entirely apparent. There was also a trend, but less regular, toward increase of pearling losses with the number of wettings.

The data on test weights (Table IV) show a decrease with the increase in amount of wetting as well as the number of wettings, but that the rate of decrease in test weight became less after the 20%–22% wettings, and also after the first wetting. There is a fair correlation between the decreases in test weight and the increases in the pearling losses.

Grades of Wheats That Were Wetted and Dried

The official grades, the percentages of dark hard vitreous kernels, and the percentages of total damage obtained by the U. S. Grain Inspection Department, Kansas City, Mo., are given in Table IX. The lowering of numerical grades corresponds closely with the decreases in test weights given in Table IV. There is also a distinct trend in changing from dark hard winter to hard winter with the amount and number of wettings. The percentages of dark hard vitreous kernels decreased very markedly with the increase in amount of wetting and to a much less extent with the number of wettings. The amount of wetting had a greater effect in decreasing the vitreous condition than the number of wettings. The reason for this has already been discussed.

Texture of Wetted and Dried Samples

The barley cutter, used on the samples treated before threshing, was used also on the samples wetted after threshing. The results of this examination are given in Table X, which gives the percentages of mealy, semivitreous, and vitreous kernels. Those kernels not definitely mealy or vitreous were designated as semivitreous.

The percentages of mealy kernels increased with the amount of wetting and with the number of wettings, and the vitreous kernels decreased similarly. The most abrupt change was at about 20% moisture. Moisture percentages lower than this have much less effect in increasing the number of mealy kernels or in decreasing the number of vitreous. The semivitreous also show a sharp increase from the 20% wetting and above. It is apparent, therefore, that in order to produce the mealy or semivitreous condition it is necessary to add enough water to produce a certain amount of swelling. To get this swelling at 20% moisture it is necessary to have more than one wetting. With several wettings the swelling that produces the mealy conditions is obtained in wetting to 20% moisture.

TABLE IX

Market Grades of Wetted and Dried Wheats

GRADES

Moisture to which wetted	Times wetted					
	1	2	3	4	5	6
%						
12	1 DHW	2 DHW	2 DHW	2 DHW	2 DHW	2 DHW
14	2 DHW	2 DHW	2 DHW	2 DHW	2 DHW	3 DHW
16	2 DHW	3 HW	3 DHW	3 DHW	3 DHW	3 DHW
18	3 DHW	3 DH	3 HW	3 DHW	3 DHW	3 HW
20	3 DHW	3 DHW	3 HW	3 DHW	4 DHW	4 HW
22	3 DHW	3 HW	3 HW	3 DHW	4 DHW	4 HW
24	3 HW	3 HW	3 HW	4 HW	4 HW	4 HW
26	3 DHW	3 HW	3 HW	4 HW	4 HW	5 HW
28	3 HW	3 HW	4 HW	4 HW	4 HW	5 HW

Check 1 DHW, TW 61.3

DARK HARD VITREOUS

%	%	%	%	%	%	%
12	92	91	97	94	98	87
14	84	87	99	96	97	95
16	88	67	84	87	96	89
18	84	87	70	88	94	70
20	81	79	73	90	77	60
22	80	28	64	76	78	60
24	65	28	27	29	42	27
26	75	46	26	28	40	27
28	27	38	49	30	27	55

Check DHV 88

TOTAL DAMAGE

%	%	%	%	%	%	%
12	0	3.0	0	0	0	0
14	0	0	1.5	0	0	0
16	3.0	3.3	0	0	1.0	0
18	1.4	2.4	1.5	1.0	0	0
20	2.3	2.0	5.0	3.0	0	5.0
22	3.8	2.5	6.0	3.0	2.0	0.7
24	0	5.2	3.0	4.2	10.0	10.0
26	0	2.0	3.0	3.5	9.4	11.0
28	0	8.0	6.7	4.3	10.0	15.0

Check total damage 0

TABLE X

PERCENTAGES OF MEALY, SEMIVITREOUS, AND VITREOUS KERNELS IN FIFTY

Moisture to which wetted	Times wetted					
	1	2	3	4	5	6
	MEALY					
%	%	%	%	%	%	%
Check	0	—	—	—	—	—
12	4	6	4	2	4	4
14	2	2	8	4	4	0
16	4	14	4	6	4	6
18	8	10	10	10	12	14
20	12	12	18	30	24	40
22	18	24	26	36	40	34
24	24	26	36	44	52	42
26	24	36	48	44	60	58
28	34	46	54	66	66	50
	SEMIVITREOUS					
%	%	%	%	%	%	%
Check	2	—	—	—	—	—
12	8	2	4	4	4	2
14	8	6	4	6	6	10
16	8	36	4	4	8	14
18	10	44	16	28	34	28
20	50	54	46	52	42	46
22	52	56	48	48	44	48
24	54	64	46	44	38	44
26	68	22	38	46	30	34
28	56	46	38	32	28	38
	VITREOUS					
%	%	%	%	%	%	%
Check	98	—	—	—	—	—
12	88	92	92	94	92	90
14	90	92	88	90	90	90
16	88	56	92	90	88	80
18	82	46	74	62	54	58
20	38	34	36	18	34	14
22	30	20	26	16	16	18
24	22	10	19	12	10	14
26	8	42	14	10	10	8
28	10	8	8	2	6	12

Baking Tests on Selected Samples

Baking tests were made on a few samples representing the various conditions of treatment both before and after threshing as indicated by the headings in Table XI, which also give the description of the samples selected for these tests and the results by two different formulas. These formulas were:

Formula I	Formula II
100 g. flour	100 g. flour
Absorption as needed	Absorption as needed
5 g. sugar	6 g. sugar
1 g. salt	1½ g. salt
3 g. yeast	3 g. shortening
0.3 g. malt syrup, 200 Lintner	2 g. yeast
0.1 g. $NH_4H_2PO_4$	4 g. dry milk solids
2 mg. $KBrO_3$	¼ g. malt syrup, 120 Lintner
	3 mg. $KBrO_3$

A third formula which was also used differed from the second only in having 4 mg. $KBrO_3$ instead of three. The results were almost duplicates of those obtained from Formula II.

The doughs were mixed to the optimum as determined by observation in the Swanson-Working type of mixer. This time varied from 3¼ minutes to 4 minutes (84 r.p.m.). The three-hour standard fermentation and proofing times were used, namely 105 minutes to first punch, 50 minutes to second punch, 25 minutes to pan. The proofing time was 55 minutes, and the baking time was 25 minutes at 430°F.[3]

The choice of the comparatively rich formulas containing milk and several milligrams of $KBrO_3$ were based on a study of baking methods for evaluating the quality of hard red winter wheat varieties (Finney and Barmore, 1939). Twenty-two different methods were included in that study.

The mixing time obtained for Formula I was constant at 3¼ minutes for all the samples. The slightly longer mixing time for Formula II was probably due in part to the presence of the shortening. However, the sample exposed to the ten rains, totaling 5.16 inches, and the one wetted six times to 28% had the longest mixing times.

The figures for loaf volume are apparently of the most significance in arriving at the baking values provided they are accompanied by satisfactory grain, texture, and crumb color. These latter do not differ significantly among the different samples and hence these characteristics were unaffected by the various treatments.

The largest loaf volumes and the highest baking scores were obtained from the sample threshed from the uncovered shock exposed to ten rains

[3] The baking tests were made by Karl F. Finney of the Hard Winter Wheat Quality Laboratory, to whom credit for this work is due.

TABLE XI

Baking Tests on Selected Samples

FORMULA I

Treatment		Mix. time	Abs.	Loaf vol.	Grain	Texture	Crumb color	Baking value	Number loaf Fig. 1
Before threshing:		*min.*	%	*cc.*	%			%	
Check		3½	59	700	83	G+VG[1]	63 cy[2]	80	1
No wetting, dried in sun		3½	58	670	82	G+VG	65 cy	77	2
Wetted 4 times, dried in shade		3½	60	703	82	G+VG	70 cy	81	3
Shock, canvas covered		3½	58	683	82	G+VG	63 cy	78	4
Shock, threshed before the rains		3½	61	723	82	G+VG	67 cy	82	5
Shock, threshed after ten rains		3¼	60	803	83	G+VG	65 cg	90	6
After threshing:									Number loaf Fig. 2
Times wetted	Per cent wetted								
1	12	3½	59	710	82	G+VG	68 cg	81	1
1	28	3½	62	740	82	G+VG	70 cy	85	2
3	22	3½	60	710	82	G+VG	68 cy	81	3
5	22	3½	61	680	82	G+VG	68 cy	78	4
6	12	3½	59	713	83	G+VG	65 cy	82	5
6	28	3½	61	690	83	G+VG	70 cy	80	6

FORMULA II

Treatment		Mix. time	Loaf vol.	Grain	Texture	Crumb color	Baking value	Number loaf Fig. 1
Before threshing:		*min.*	*cc.*	%			%	
Check		3½	748	94	VG	82 cr[2]	91	7
No wetting, dried in sun		3½	725	93	VG	82 cr	89	8
Wetted 4 times, dried in shade		3½	768	94	VG	86 crg	93	9
Shock, canvas covered		3½	755	90	VG	77 cr	85	10
Shock, threshed before the rains		3¾	783	90	VG	84 cg	94	11
Shock, threshed after ten rains		4	995	93	VG	88 cw	117	12
After threshing:								Number loaf Fig. 2
Times wetted	Per cent wetted							
1	12	3½	753	93	VG	83 cg	91	7
1	28	3½	805	92	VG	87 cg	97	8
3	22	3½	790	94	VG	84 cr	96	9
5	22	3½	778	94	VG	84 cr	94	10
6	12	3½	765	94	VG	84 cr	93	11
6	28	4	863	94	VG	85 cr	104	12

[1] G = good. VG = very good.
[2] cy = creamy yellow; cg = creamy gray; cr = creamy; cw = creamy white.

totaling 5.16 inches and from the sample wetted as grain six times to 28% and dried between wettings, except with Formula I; the latter sample gave no better values than the other samples wetted as grain. For all the other samples the loaf volumes and the baking values were not significantly correlated with the treatments either as grain or before threshing. Larger loaf volumes were obtained from all samples with Formula II than from Formula I.

Photographs of the loaves baked with Formulas I and II are shown in Figures 1 and 2. Figure 1 shows the loaves from the samples treated before threshing, and Figure 2 shows those from the samples wetted as grain. Loaves 1 to 6 in each figure were baked by Formula I and loaves

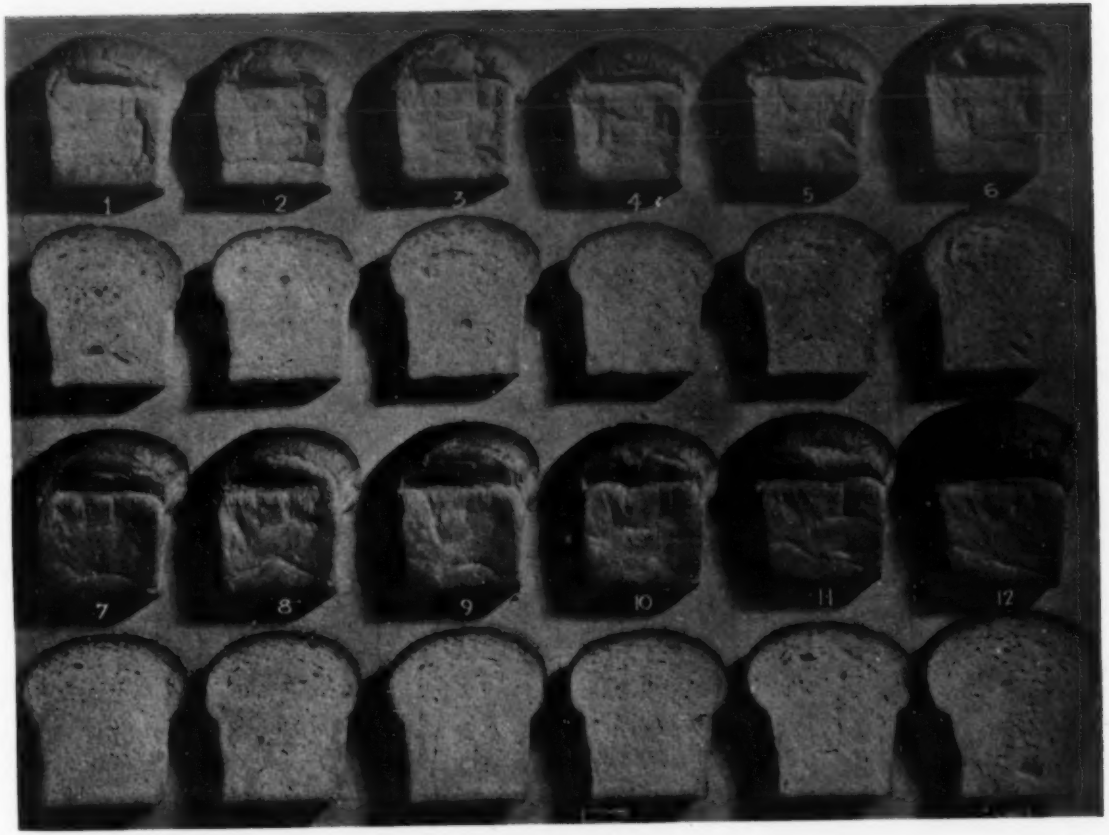

Fig. 1. Samples treated before threshing.

Loaf numbers 1 & 7, checks; 2 & 8, no wetting, dried in sun; 3 & 9, wetted 4 times, dried in shade; 4 & 10, canvas covered shock; 5 & 11, threshed from shock before rain; 6 & 12, threshed from shock after ten rains.

Loaves 1 to 6 baked by Formula I, 7 to 12 by Formula II.

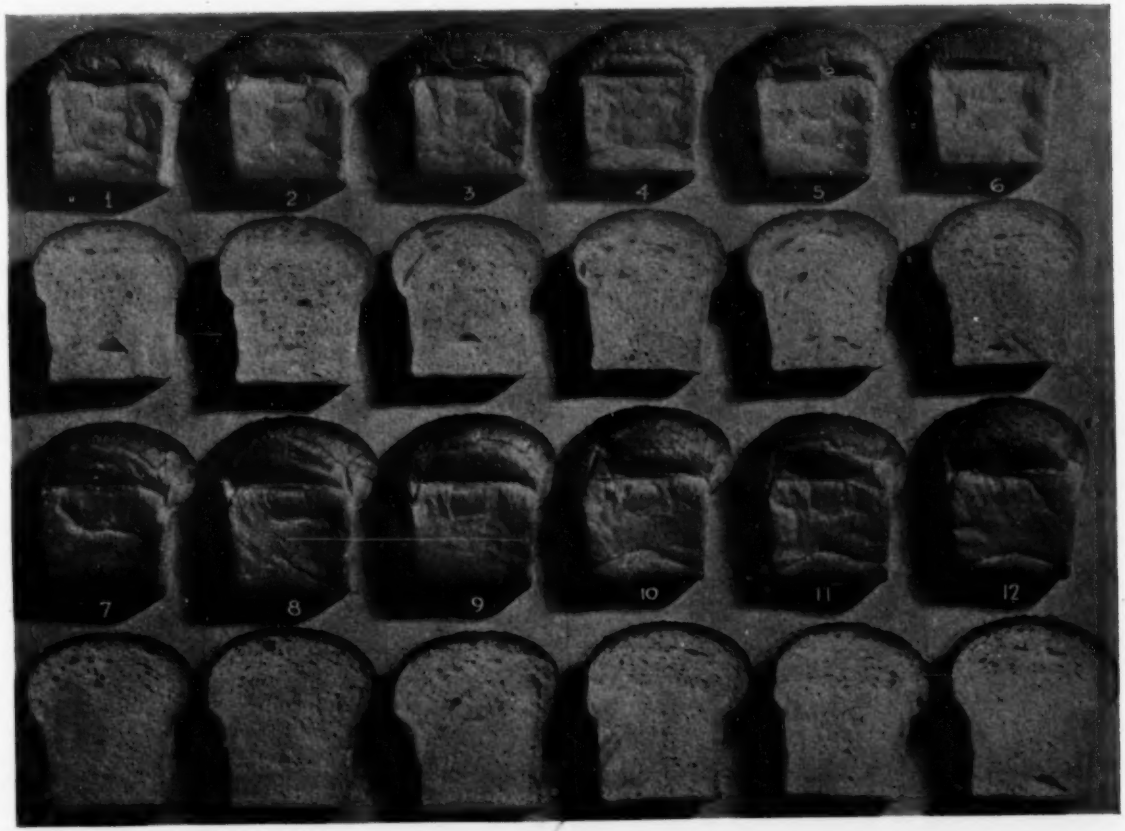

Fig. 2. Samples treated after threshing.

Loaf numbers 1 & 7, wetted once to 12%; 2 & 8, once to 28%; 3 & 9, three times to 22%; 4 & 10, 5 times to 22%; 5 & 11, 6 times to 12%; 6 & 12, 6 times to 28%.

Loaves 1 to 6 baked by Formual I, 7 to 12 by Formula II.

7 to 12 by Formula II. The photographs show the uniformly good grain and texture obtained regardless of treatment.

Improvement from the Process of Aging

Here is a singular situation of larger loaf volumes and baking scores obtained from the two samples which had had the most severe treatment and consequent lowest grain-grading value. The shock sample threshed after the ten rains graded No. 5 sample-grade hard winter with a total damage of 16.4% (Table II), and the sample wetted six times to 28% graded No. 5 hard winter with a total damage of 15% (Table IX). In contrast to these, the sample dried in the sun and not wetted by rains and the sample wetted once to 12% both graded No. 1 dark hard winter with no damage. The loaf volumes of the samples graded with no damage were 247 cc. and 110 cc. smaller, respectively, than those which graded as damaged.

That the process of germination, if not carried too far, will increase loaf volume was shown by Willard and Swanson (1911). The loaf volumes with good textures obtained when wheat was placed under conditions of germination for 24, 48, and 72 hours were 83, 88, and 93 cubic inches respectively as compared with 85 cubic inches for the check loaf. When the germination was for longer periods, the loaf volumes were decreased and the texture was very poor. Swanson and Fenton (1933) obtained an improvement in loaf volume and texture by heating new wheat at 40°C. for as much as nine days. Heating at 45°C. produced improvement up to five days of heating, but longer periods produced deterioration. That larger loaf volumes and better texture are obtained from wheat which has had a limited amount of exposure to wetness than from new wheat which has not been so exposed seems to be a common observation.

The wheat kernel is a living organism and as such is continually undergoing change. Before flour-bleaching methods were perfected and the use of bromate introduced, flour from new wheat was much more of a problem than now. That the loaf volumes will be larger after wheat has aged was shown by Fitz (1910) who reported 2,440 cc. volume from shock-threshed wheat and milled at once, and 2,710 cc. from wheat stacked and then milled 57 days after harvest. However, if wheat is stored too long there will be a deterioration. Stockham (1920) reported that wheat stored in an elevator in North Dakota showed a significant increase in baking value during the first year. During five years thereafter there was little change in samples successively milled, but after six years there appeared a decrease in quality. Too long storage, no matter if under dry conditions, will bring deterioration. A sample of wheat

known to be about 25 years old gave a loaf volume of 1,140 cc. as compared with 2,090 for normal wheat (Swanson, 1937). The samples which gave larger loaf volumes and greater baking values, shown in Table XI, and which were graded as having 15% and 16.4% damage respectively, had attained the maximum beneficial change due to induced aging. That these two samples which gave the largest loaf volumes and the highest baking score would deteriorate in storage much more rapidly than the others which showed no damage may be inferred from existing information.

Recording Dough Mixer Curves from Representative Samples

A few curves from representative samples were made on the recording dough mixer. Unfortunately not enough flour was left to make the curves on the same flours which were used in baking, but those presented in Figure 3 show effects of representative treatments. Curves 1

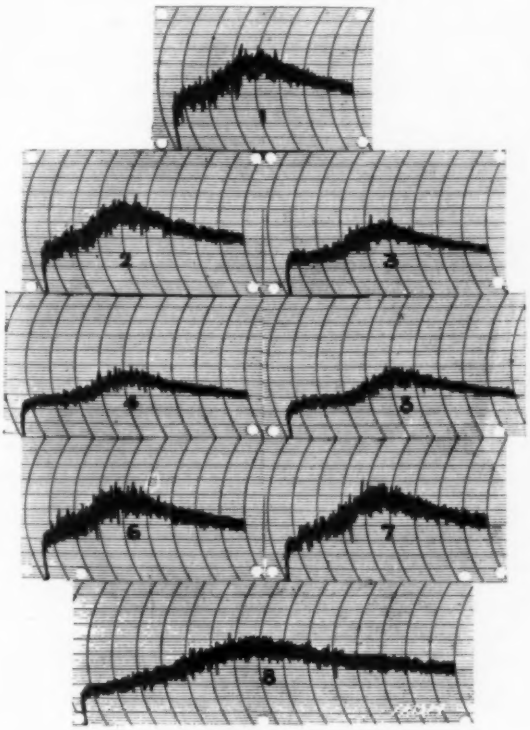

Fig. 3. Curves from representative samples.

1. Check. Field threshed grain—no wetting.
2. Threshed from shock before rains.
3. Threshed from shock after 10 rains, 5.16 inches.
4. Threshed from outside bundles of capped shock.
5. Threshed from outside bundles of uncapped shock.
6. Grain wetted once to 26%.
7. Grain wetted three times to 24%.
8. Heat-damaged flour.

and 2 represent the pattern obtained when the wheat had suffered no damage. Curves 3, 4, and 5 show the pattern when there were increasing amounts of damage. Curve 7, from wheat wetted three times to 24%, had a notably longer development time than Curve 6, which was from wheat wetted once to 26% indicating an effect similar to that obtained when the wheat was exposed to rains. Curve 8 was made from a badly heat damaged flour and shows that one effect of damage is to increase the dough development time. Curve 3, from the average of the whole shock, does not show as much deviation from the pattern of the check curve as Curves 4 and 5, which were from wheat taken from the outside of shocks exposed to the ten rains totaling 5.16 inches. On the whole the patterns of these curves show greater differentiations due to treatments than do the baking results.

Summary

Results from exposing wheat before threshing to various amounts of moisture and also from wetting threshed wheat grain to various moisture percentages from one to six times have been presented.

Wetting wheat artificially in the straw and then drying did not produce as great physical effects as when shocked wheat was exposed to several rains. Wetting as grain produced changes somewhat similar to those caused by exposure to the rains.

The greatest apparent effects of wetting wheat were a lowering of test weight and decreases in the vitreous condition. The hardness as determined by the barley pearler was also decreased. Since these properties are important factors in grain grading, wetting wheat either artificially or by exposure to rain or by adding water to the grain and then drying did seriously affect the grading values of the grain.

The decreases in test weight were not reflected in correspondingly lower flour yields in the samples wetted after threshing. The samples wetted as grain, the test weights of which had been reduced as much as six pounds, gave essentially as high flour yields as the samples not wetted or those wetted comparatively little. This was not due to closer milling, since the ash figures varied only within the experimental limits.

Decrease in test weight is due mostly to the swelling of the kernels and partly to the roughening of the bran coat. Because there was no loss of material, there should have been no decrease in flour yield simply because the space occupied by the kernels in the test kettle had been increased. Repeated wetting may have facilitated separation of bran from the endosperm.

The baking values obtained on representative samples did not correlate with the commercial grade of the grain nor with the severity of the

treatment. An explanation for this situation is offered. The curves made on the recording dough mixer showed a longer time of development and a decrease in height as a result of the more severe treatments.

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DEVELOPMENT OF *B. MESENTERICUS* IN BREAD AND CONTROL WITH CALCIUM ACID PHOSPHATE OR CALCIUM PROPIONATE

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Many factors are known to influence " rope " development in bread. Some of these are the temperature of bread storage (Watkins, 1906), the pH of the bread (Lloyd and McCrea, 1918; Cohn *et al.*, 1918), and methods of bread baking (Amos and Kent-Jones, 1931). The possible sources of infection have received considerable attention, culminating in a tentative standard spore count of bread ingredients by Hoffman, Schweitzer, and Dalby (1937).

The technique of study of " rope " in bread has varied considerably, and our knowledge concerning several of the factors is quite general rather than reproducible by experimentation. One of the variables in experimental technique that has been handled in quite different manners by various authors has been the method of inoculation of experimental bakes. Watkins (1906) used 1 to 20 ml of peptone wort culture. Fisher and Halton's (1928) procedure reads, " The liquor used in making the loaves was heavily inoculated with a water suspension of ropy bread." Amos and Kent-Jones (1931) used flour containing " rope " spores. Cohn *et al.* (1918) inoculated bread with 1 ml of a culture per loaf. Most of these experimenters did not determine the number of bacteria that were added to the dough.

The authors of the present paper conducted experimental bakes with determined numbers of bacteria for inoculum, hoping to eliminate one of the uncontrolled variables of previous studies. The present paper deals with investigation of the following points: (1) a study of the amount of inoculum necessary to produce a " ropy " condition in finished bread, (2) the effect of pH of the bread on development of " rope," and (3) a comparison of calcium acid phosphate and calcium propionate as inhibitors of *B. mesentericus* in bread.

Experimental Method

Cultures used: All cultures used in this study were originally obtained from " ropy " bread or from ingredients being used in bakeries experiencing " rope." These cultures were isolated through the use of the technique of Hoffman, Schweitzer, and Dalby (1937) for quantitative analysis of bread ingredients, and were purified by serial plating on

various media. All cultures used were spore-forming rods conforming in general to the morphological description of *Bacillus mesentericus* Trevisan as given in Bergey's manual (1939).

Table I contains data on the source of the cultures and some growth characteristics. Hoffman has used the formation of a pellicle on nutrient broth as a distinguishing character for *B. mesentericus*. Bergey's manual, in describing this organism, states: "Broth: turbid becoming clear, with or without fragile pellicle." As shown in Table I, culture *R* when first isolated caused turbidity but did not form a pellicle. In later tests, culture *R* did display this ability. Culture *Q*, on the other hand, formed a good pellicle when first isolated but lost the property of pellicle formation temporarily at a later date. From an examination of Table I, it is evident that there is considerable variability in pellicle formation by

TABLE I

CULTURAL CHARACTERISTICS OF *B. MESENTERICUS* STRAINS USED IN STUDY

Culture	Source	Pellicle (nutrient broth)			Pellicle (potato-nutrient)
		Test 1	Test 2	Test 3	
A	Malt	?	Poor	Poor	Good
B	Bread	?	Fair	Fair	Good
G	Bread	Good	Fair	Poor	Fair
M	Rye flour	Fair	Fair	Poor	Good
N	Rye flour	Poor	Fair	Fair	Good
Q	Malt	Good	None	Fair	Fair
R	Malt	None	Poor	Poor	Fair
S	Yeast	Fair	Poor	Poor	Fair
V	Rye flour	Good	Poor	Poor	Fair
X	Bread	Good	Fair	Poor	Fair
Y	Bread	Good	Poor	?	Poor

cultures at various dates. Perhaps nutrient broth on which these tests were conducted is not the proper medium for displaying this character. A test in which two-thirds nutrient broth was combined with one-third potato decoction showed better pellicles than on nutrient broth alone.

The cultures varied from white to red or brown in the pigment produced on potato dextrose agar. The pink coloration formed by cultures *N* and *Y* are especially striking. These are probably similar to the variety "ruber" described by Kent-Jones and Amos (1930).

At the beginning of the study, single strains were used in various bread bakes. The first strains studied, *A* and *B*, were slow in developing the symptoms of "rope" in the bread. The development of viscousness was especially slow, usually requiring 9 to 10 days at 90°F. Strains *A* and *B* had been in culture on potato dextrose medium for about one year and may have lost their virulence.

All of the 11 cultures were then tested for virulence by inoculating sterile slices of bread and incubating these slices at 37°C. The most virulent strains were then used in combinations in later bakes. The combination *G-S-X*, which developed the symptoms most rapidly and consistently, was used for most of the studies.

The mixed culture *G-S-X* produced a "ropy" odor in bread in 1½ to 3 days, a brown discoloration in 3 to 5 days, and viscousness in 3 to 6 days when the inoculum was greater than 100,000 per loaf (368 per gram of flour). The storage temperature of the bread was 90°F (32°C) and the relative humidity 85%–88%. The effect of a smaller inoculum is described in a later portion of the paper. For most of the bakes the inoculum used was bread which had been infected with the mixed culture G-S-X. This bread, after development of "rope," was dried and powdered. The powder was thoroughly mixed and then plated to obtain a value for spores per gram of the powder. By using various amounts of dry powder, it was possible to inoculate the dough with a definite number of spores. A weighed amount of powder was suspended in 9 ml of sterile distilled water, allowed to stand for at least a half hour with intermittent, vigorous shaking. Two ml of this suspension or a dilution of it was added to 50 ml of the water for a five-loaf batch of dough. The inoculum was added to the sponge during mixing. The amount of inoculum added to any batch was double checked by platings made at the time of inoculation of the dough.

Method of baking: The bread used in this study was baked by a modified sponge and dough method, the formula for which was as follows (percentages based on weight of flour):

Sponge		%
Flour	815 g	60
Water	610 g	45
Yeast	27–35 g	2.0–2.6
Yeast food	5.4 g	0.4

Dough		%
Flour	543 g	40
Water	350 g	25.8
Salt	25 g	1.8
Sugar	79 g	5.8
Malt	11 g	0.8
Shortening	45 g	3.3
Milk powder	27–108 g	2–8

The flour used was a commercial baker's brand, southeastern white flour containing 12% protein and 0.41% ash. The flour and malt used in the bakes were tested for "rope" organisms. None were found in the flour but the malt contained 10 spores per gram. No counts were conducted on the yeast which was purchased at intervals. The sponge was held 4.5 hours at 80°F (27°C) and proofing time was 30–35 minutes. The bread was baked 35 minutes at 405–410°F in a Lydon experimental oven. Each baking experiment consisted of three to four doughs producing five loaves each.

Storage of test bread and collection of data: After baking, loaves were cooled at room temperature and then heat sealed in moisture proof "cellophane," cellulose film. The bread was left unsliced. It was then stored in a cabinet with temperature controlled at 90°F (32°C) and relative humidity of 85%–88%.

The values for pH were obtained about 17 hours after baking. Measurements were made with a Beckman pH meter. The delay in taking pH may account for slight discrepancies in later tables since Fisher and Halton (1928) have shown an increase in pH of bread due to growth of *B. mesentericus.*

There were always five loaves of bread to a treatment. The first loaf was examined for "rope" symptoms at the end of 24 hours of storage. The first symptom displayed was the development of a "ropy" odor. This was followed by development of brown discoloration of the crumb, and later viscousness of the crumb. It was found that the same loaf of bread could be used for several days' examination if it were again sealed properly in "cellophane" before returning to storage.

Experimental Results

Effect of varying amounts of inoculum: Hoffman, Schweitzer, and Dalby (1937) established the following tentative standards of objectionable "rope" counts for bread ingredients: "Counts of 20 per 100 g of flour, 100 per gram of yeast or malt and 10 per gram of other ingredients." Using these counts for flour, yeast, and malt only, bread baked according to the authors' experimental formula should not contain over 814 bacteria per loaf, or about three per gram of flour. Table II gives the results of bakes containing varying amounts of inoculum per loaf and gram of flour.

TABLE II

EFFECT OF AMOUNT OF INOCULUM ON THE DEVELOPMENT OF "ROPE"

Bacteria per loaf	Bacteria per gram flour	pH of bread	Days for development of:		
			Odor	Color	Viscousness
4,000,000	14,000	5.50	1.5	3	3
380,000	1,300	5.52	2	3	3
26,000	96	5.53	3	4	5
15,000	55	5.58	3.5	4	7[1]
1,600	6	5.58	6	7[1]	8[1]
170	0.6	5.57	10+	10+	10+

[1] In isolated spots only, not general throughout crumb.

The results of this test offer confirmation of Hoffman's criterion of safe counts of bacteria in the dough ingredients. With an inoculum of

1,600 bacteria per loaf, or 6 per gram of flour (about twice the value of Hoffman's standards for counts on flour, yeast, and malt), "rope" symptoms developed slowly under storage conditions of elevated temperature and humidity. "Rope" did not develop even after ten days of such storage when the inoculum was 170 bacteria per loaf, or about one-fifth Hoffman's criterion. As the inoculum was increased, the rate of development of symptoms also increased. Above 380,000 bacteria per loaf an increase in the inoculum did not reduce markedly the time of development of "rope" symptoms with this method of experimentation.

Effect of pH of bread on the development of "rope": Several authors (Lloyd and McCrea, 1918; Cohn *et al.*, 1918; and Morison and Collatz, 1921) have reported that the limiting hydrogen ion concentration for the growth of *B. mesentericus* lies only a few tenths of a pH below the usual pH values of commercially baked breads. Tests on the pH value of 32 brands of bread purchased in the open market show values ranging from pH 5.22 to 5.82, with an average of pH 5.50.

The recent trend toward increasing milk solids in commercially baked breads is tending to increase the pH values of the bread, and it is possible that the average pH for all breads will be increased in the future.

The present authors were interested in learning if there was variability in the rate of development of "rope" in breads varying in pH from 5.3 to 5.8. By varying the amounts of milk solids and yeast in the experimental doughs, it was possible to vary the pH of the final bread from 5.36 to 5.84.

Breads of these varying pH values were inoculated in the usual manner with "rope" bacteria. In one test the inoculum used was 260,000 bacteria per loaf (960 per gram of flour). In the other test one-twenty-seventh of this number or 9,600 bacteria per loaf were used (35 per gram of flour). Table III gives the results of these two tests. When the high inoculum was used, the difference in the rate of development of "rope" symptoms at the three pH levels was very small. The only symptom which varied to any extent was the development of viscousness. At pH 5.8 viscousness developed in three days, while at pH values below this it required four days.

With only 9,600 bacteria per loaf, a greater difference becomes apparent at the lower pH value. A half day longer was required for the development of odor, two days for color, and three days longer for viscousness at pH 5.37 than was required at pH 5.57. Above pH 5.57 there was little difference except that a half day less was required for odor development at pH 5.87. The results of these two tests show only slight differences in development of symptoms from the results given in Table II for comparable inoculum and pH of bread.

TABLE III

EFFECT OF BREAD pH ON DEVELOPMENT OF "ROPE"

Original pH of bread	Milk solids used	Yeast used	Bacteria per loaf	Days for development of:		
				Odor	Color	Viscousness
	%	%				
5.84	8.0	2.0	260,000	2.5	2.5	3
5.70	6.0	2.2	260,000	2.5	2.5	4
5.36	2.5	2.6	260,000	2.5	2.5	4
5.87	8.0	2.0	9,600	3.5	5.0	6
5.69	5.0	2.2	9,600	4.0	5.0	6
5.57	4.0	2.6	9,600	4.0	5.0	6
5.37	2.0	2.6	9,600	4.5	7.0[1]	9[1]

[1] In isolated spots only, not general throughout crumb.

Inhibitory effect of calcium acid phosphate: Calcium acid phosphate has been used for a number of years in commercial bread baking as a means of controlling the development of *B. mesentericus*. The usual recommendation for the amount of this material used is 0.20%–0.25%. (If symptoms of "rope" develop, double these amounts are recommended.) Fisher and Halton (1928) compared the effect of several acidic ingredients on the development of "rope" and found that calcium acid phosphate possessed greater inhibitory action than did phosphoric acid itself. They attributed this surprising result to the better distribution of the calcium salt than of the acid during mixing of the dough, with a correspondingly more even distribution of pH throughout the crumb of the bread produced. With an equivalent lowering of pH, calcium acid phosphate was found more effective than tartaric acid, but no satisfactory explanation of this fact was made. Fisher and Halton, nevertheless, came to the general conclusion that the inhibitory action of calcium acid phosphate is mainly due to lowering the pH.

The present studies included the effect of calcium acid phosphate on the final pH of the baked bread. Bakes were made using the same concentration of phosphate in breads of different pH values. As previously mentioned, the different pH values were obtained by varying the amounts of milk solids and yeast.

With the flour used, the effect of any one concentration of calcium acid phosphate in lowering the pH was about the same, irrespective of the pH of the untreated bread. Thus 0.25% of calcium acid phosphate lowered the pH about 0.14, whether the untreated bread had a pH of 5.85 or 5.44. Using 0.5% of calcium acid phosphate, the pH was lowered in the range of 0.34. Fisher and Halton have shown that a final pH of phosphate-treated bread of about 5.2 is required for inhibition of the "rope" organism. Thus the pH of the bread without phosphate

would have to lie in the range of 5.35 if appreciable inhibition were to be obtained through the use of 0.25% of calcium acid phosphate.

In order to obtain a proper evaluation of the effect of calcium acid phosphate as an inhibitor, it was believed that tests in which the amount of inoculum was varied would be necessary for a basis of comparison. Accordingly, 0.25% of calcium acid phosphate was used in a test bake in which varying amounts of bacteria were added to the sponge.

Table IV gives the results of this test, indicating " days for appearance " of the various symptoms and the " days of inhibition." The latter were computed by subtracting the " days for appearance " of Table II from Table IV at approximately equal levels of inoculum.

TABLE IV

"ROPE" INHIBITION OBTAINED WITH 0.25% CALCIUM ACID PHOSPHATE IN BREAD CONTAINING VARYING AMOUNTS OF INOCULUM

Bacteria per loaf	pH of bread	Days for appearance of:			Days of inhibition of:		
		Odor	Color	Viscousness	Odor	Color	Viscousness
4,900,000	5.31	5	6	8	3.0	3	5
490,000	5.34	6	7	9	3.5	4	6
37,000	5.36	8	10	10	5.0	6	5

Considering the time required for the appearance of various symptoms, it is evident that the amount of inoculum greatly influences the effect of calcium acid phosphate in retarding " rope " development. The more bacteria, the less effective is the phosphate.

It is also apparent from Table IV that 0.25% phosphate is effective in bread with a final pH above 5.2, if the inoculum is relatively low. The inhibition produced was better than that obtained by Fisher and Halton, who undoubtedly used higher inoculum.

To further test the effect of calcium acid phosphate as a " rope " inhibitor, a series of bakes were made using varying amounts of phosphate in breads with different pH values and varying inoculum. The amount of inoculum in all cases was relatively high. In most bakes, controls with inoculum but without phosphate were tested. The variations in symptom development for controls were as follows: odor 1.5–3 days, color 3–5 days, viscousness 3–6 days.

The results of several tests are compiled in Table V. Conclusions which may be drawn from this table are:

1. Calcium acid phosphate at 0.5% produces effective inhibition of " rope " in bread the pH of which would be 5.84 without the phosphate and with the phosphate 5.40. Other investigators, who state that the

pH value of the phosphate-treated bread must be 5.20 or lower for effective inhibition, probably used very high numbers of bacteria.

2. A phosphate concentration of 0.375% is an effective inhibitor in breads of final pH value of 5.4 or lower.

3. A concentration of 0.25% phosphate has little retarding effect in breads of high pH, but as the pH of the bread is lowered more effect is observed.

TABLE V

EFFECT OF VARYING AMOUNTS OF CALCIUM ACID PHOSPHATE ON "ROPE" DEVELOPMENT

Amount of phosphate	pH of untreated bread	pH of treated bread	Bacteria per loaf	Days for appearance of:		
				Odor	Color	Viscousness
%						
0.25	5.80	5.70	360,000	3	4	4
0.25	5.60	5.50	360,000	5	7	9+
0.25	5.50	5.36	490,000	6	7	9
0.25	5.45	5.25	2,360,000	6	6	11
0.375	5.59	5.40	340,000	8	9	11+
0.375	5.43	5.18	1,640,000	8	11+	11+
0.5	5.84	5.40	340,000	9	9	10+
0.5	5.59	5.21	340,000	11+	11+	11+

Inhibitory effect of calcium propionate: The salts of propionic acid, especially calcium and sodium propionate, have been used for several years as mold inhibitors for bread and bakery products (Pyler, 1938). Weimershaus and Svenson (1938), using propionic acid rather than calcium propionate, found 0.25% to completely prevent "rope." The acid-treated bread required longer fermentation time and the volume of the bread was less than the untreated. The investigators gave no details on experimental methods. Commercial baking experience with calcium propionate has shown this material to be an effective "rope" inhibitor, but concentrations which are effective for inhibition under various conditions have not been reported in the literature.

The inhibitory action of propionates does not depend on lowering the pH of the bread as in the case of the calcium acid phosphate. Hoffman, Schweitzer, and Dalby (1939) have shown that the acids of the acetic series up to lauric are inhibitory to fungi when used in a medium with a pH value below 7.

Breads baked with varying amounts of propionate salts did not show a consistent change in pH from that of the control bread of the same bake. It was usually found that the pH value of the propionate-treated bread was a few hundredths lower than that of the control bread. Often,

however, the reverse was true. Of 32 bakes, only three showed greater variation in pH than 0.05 from the controls. No correlation was found between the amount of propionate salt added and the resulting pH variation from the control bread. Unlike calcium acid phosphate, the propionates exhibited inhibitory action without markedly changing the pH of the bread.

The effect of varying amounts of inoculum on inhibition of "rope" in bread containing 0.11% of calcium propionate was studied in a manner similar to that used in the study of calcium acid phosphate. Table VI

TABLE VI

"ROPE" INHIBITION OBTAINED WITH 0.11% CALCIUM PROPIONATE IN BREAD CONTAINING VARYING AMOUNTS OF INOCULUM

Bacteria per loaf	pH of bread	Days for appearance of:			Days of inhibition of:		
		Odor	Color	Viscousness	Odor	Color	Viscousness
9,700,000	5.54	5	7	8	3.5	4	5
980,000	5.53	8	11	13	6	8	10
72,000	5.54	12	13	13	9	9+	8+

gives the results of this study. Unfortunately the inoculum for this test was about double that given in the study of calcium acid phosphate in Table IV. However, by interpreting Tables IV and VI, and considering results obtained in Table II, it is possible to draw a fair correlation.

It is first of all evident that 0.11% of propionates is more effective than 0.25% of calcium acid phosphate at all levels of inoculum in bread of approximately similar original pH. Propionates apparently delay the development of color and viscousness more than does calcium acid phosphate. Thus in Table IV there was a spread of only two to three days between the time of odor and viscousness development with calcium acid phosphate, while this spread was three to five days with calcium propionate.

Table VII gives the results of bakes with various amounts of calcium propionate at different pH levels. The authors believe the following conclusions can be drawn from Table VII:

1. Calcium propionate at 0.188% produces effective inhibition of "rope" in bread the pH of which is as high as 5.8. At pH 5.6 a concentration of 0.156% will produce as good inhibition.

2. At 0.11% calcium propionate produces considerable inhibition even when the pH of the bread is quite high and the retarding effect becomes more marked as the pH is lowered.

3. Lower concentrations of calcium propionate are also somewhat effective if the pH of the bread is reduced. Thus as little as 0.075% calcium propionate shows some retarding action in bread of pH 5.4.

TABLE VII

EFFECT OF VARYING AMOUNTS OF CALCIUM PROPIONATE ON "ROPE" DEVELOPMENT

Amount of propionate	pH of untreated bread	pH of treated bread	Bacteria per loaf	Days for appearance of:		
				Odor	Color	Viscousness
%						
0.075	5.40	5.40	320,000	5	6	7
0.11	5.80	5.69	15,000,000	6	8	9
0.11	5.66	5.66	1,220,000	10	10	11
0.11	5.55	5.54	980,000	8	11	13
0.156	5.60	5.60	560,000	12	12+	12+
0.156	5.45	5.43	2,400,000	10	11+	11+
0.188	5.84	5.83	340,000	9	9	10+
0.188	5.50	5.52	440,000	12+	12+	12+

Discussion

A great hindrance to the advancement of our knowledge of the development of "rope" in bread is the lack of opportunity to study the trouble under bakery conditions. No baker wants tests conducted in his plant with these bacteria, and when an outbreak of rope occurs every effort is directed toward elimination of the conditions producing the disease or toward corrective measures.

One of the vexing questions in connection with the trouble is the part played by the inoculum from ingredients and the part played by inoculum from contaminated equipment. Ashby and Prickett (1938) have pointed out the danger of contaminated piping connecting tank scale and mixer. Such contamination undoubtedly accounts for sporadic outbreaks of "rope." What the amount of inoculum under these conditions may be is unknown, but judging from the rapid deterioration of the bread in many of these epidemics it must be very high.

Hoffman, Schweitzer, and Dalby (1937) have given some data on maximum spore counts of bread ingredients studied over a period of several years. The maximum for flour was 150 spores per gram, for yeast 20,000 per gram, for malt 10,000 per gram, and as high as 100,000 in other malted materials. Using these maxima for flour, yeast, and malt, it would be possible to introduce an inoculum of 200,000 spores per loaf according to the formula used in the present study. This is less than the inoculum used in many tests reported in this paper, and is probably also less than that used by investigators who inoculated by means of "ropy" bread or spore suspensions.

Amos and Kent-Jones (1931) reported "ropy" bread produced with less than ten spores per gram of flour while breads baked with other

flour containing 160 spores per gram failed to become "ropy." From these results the authors concluded that baking practices were of more importance than the inoculum of the ingredients. We feel that our studies show the amount of inoculum to be of greater importance than supposed by Amos and Kent-Jones. With high inocula, the effect of changing baking practice is greatly reduced as is shown in Table III. Also the effect of corrective measures is reduced as is shown in Tables IV and VI.

Summary

The development of "rope" in bread was studied by means of laboratory bakes in which the number of bacteria used for inoculum was a controlled factor.

"Rope" symptoms developed with increasing rapidity as the inoculum was raised from 6 to 1,300 bacteria per gram of flour. Larger inoculum than 1,300 bacteria per gram did not materially increase the rate of development of "rope."

Hoffman and his associates proposed standards for bacterial counts of bread ingredients. Bread baked with six bacteria per gram of flour (twice Hoffman's standard adjusted to our formula) developed symptoms very slowly. At one-third Hoffman's standard, no "rope" developed in ten days.

The pH of bread did not influence the rate of "rope" development if the inoculum used was high. With lower inoculum, the rate of development varied markedly when pH 5.37 was compared with pH 5.87.

The inhibitory effects of both calcium acid phosphate and calcium propionate were found to be influenced by the amount of inoculum used and by the pH of the bread.

Calcium propionate was found to be two to three times as effective as calcium acid phosphate. The difference in retarding effect between the two "rope" inhibitors was greatest at high pH value.

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THE STARCH DEGRADING PROPERTIES OF BARLEY MALTS[1]

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The ability of cereal amylases to hydrolyze starch and dextrins is largely responsible for their extensive use in industry. Many amylase studies have been carried out with regard to the utilization of malts in the brewing and distilling industries, and in flour technology. In addition, malt amylase finds applications in the preparation of special dextrins and sugars, in the determination of starch, in the removal of undesirable starch and dextrins from materials such as sorgo juice (Walton, Ventre, and Byall, 1940), and elsewhere. It is obvious that the most advantageous use of malt in such diverse fields must be accompanied by a complete understanding of the nature and amount of amylase in the malt.

It is generally accepted that malt amylase has three important functions in starch hydrolysis, namely saccharification, dextrinization, and liquefaction. Two components, alpha- and beta-amylase, acting independently or in combination, are recognized as performing these functions. Present knowledge indicates that alpha-amylase is essentially a liquefying and dextrinizing amylase, while the beta component is the

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major factor promoting saccharification. However, with unmodified malt extract, both saccharification and dextrinization are the result of the combined actions of the two components. On the other hand starch liquefaction has been proposed as a measure of alpha-amylase even in the presence of beta-amylase (Blom, Bak, and Braae, 1937; Józsa and Johnston, 1935; Hollenbeck, 1939). Space does not permit a complete review of the literature dealing with the properties and actions of the malt amylases. The reader is referred to reviews such as those by Hanes (1937), Hesse (1934), Lüers (1936), van Klinkenberg (1934), and Blish, Sandstedt, and Kneen (1938), and to two recent papers by Sandstedt, Kneen, and Blish (1939), and Kneen and Sandstedt (1941).

A malt well adapted to the performance of a specific function has frequently been found deficient when applied under other circumstances. Lack of methods for absolute differentiation between the amylase components has hindered any adequate estimation of the various amylolytic potentialities of malt. Methods proposed by Sandstedt, Kneen, and Blish (1939) and Kneen and Sandstedt (1941) have made practical the accurate determination of alpha- and beta-amylase activities[3] in the presence of each other. Using these and other methods a study has been made of the starch-degrading properties of twelve barley malts.[4]

Methods

"Free" extract: The malt grain was ground finely (83% passing through a 1-mm sieve, 42% through a 0.5-mm sieve) and 1 g of meal extracted with 100 ml of water for 1 hour at 30°C. After 5 minutes of centrifuging the extract was poured rapidly through cotton and preserved with toluol. The only exception to this procedure was in the preparation of the extract used for raw starch hydrolysis; here extracts 5 times as strong were used.

In connection with the preparation of the malt extract the question arose as to the degree of standardization necessary in the grinding procedure and in the ratio of meal to extraction liquid. Sandstedt, Kneen, and Blish (1939) reported that there was considerable latitude allowable in the grinding of malts previous to securing an extract for alpha-amylase determination. For the present study a barley malt was ground to 5 different degrees of fineness by a burr mill. To indicate variation in fineness the amount passing through a 0.5-mm sieve was determined for each of the meals. Progressing from finest to coarsest grind these amounts were respectively 42%, 36%, 32%, 27%, and 23%. Extrac-

[3] It should be emphasized that throughout this communication the terms "beta-amylase activity" and "alpha-amylase activity" are used to designate the actions of these specific components. In the past these terms have frequently been used incorrectly to signify the sugar producing and dextrinizing powers (both due to combined alpha- and beta-amylase action) of malt extracts.

[4] The barley malts were kindly supplied by Dr. Allan D. Dickson of the Wisconsin Agricultural Experiment Station; this cooperation greatly facilitated the study.

tions were made and saccharogenic units (Kneen and Sandstedt, 1941) determined. The values found were respectively 19.5, 19.4, 19.7, 19.2, and 19.6 units. This variation is well within the limits of experimental error. To investigate the effect of varying the proportion of malt to extraction liquid 1, 2, 3, and 4 g of a low diastatic malt were extracted in the usual manner with 100 ml of water. Saccharogenic activities were determined and unit values of 8.0, 8.0, 7.9, and 8.0 were found. This deviation likewise is insignificant. Obviously then, when determining amylase activity, considerable latitude is permissible in the preparation of malts for extraction and in the ratio of meal to extraction liquid.

"Total" extract: To 4 g of the finely ground meal 0.4 g Merck's papain was added and extraction made with 100 ml of dilute calcium acetate solution (1 g calcium acetate per liter) for 18 hours at 30°C. Toluol was used as a preservative. After centrifuging and filtering the extract was diluted 1 to 4 with water before use. The papain had adequate activity in this concentration. Increasing the ratio of papain to meal or treatment with papain "activators" gave no increase in amylase extraction.

Malt saccharogenic activity (method of Kneen and Sandstedt, 1941): Using 20 ml of buffered 2% Lintner starch, plus extract and water sufficient to give a total volume of 30 ml, the degree of saccharification taking place in 15 minutes was determined and *malt saccharogenic units* (the number of grams of soluble starch converted to maltose by 1 gram of malt in 1 hour at 30°C) were calculated. Such precautions as preparing the starch and calculating the units on a dry weight basis, and subtracting a starch-extract "blank" were observed. A convenient method for determining the starch-extract blank is to add to the starch and water in the reaction flask the 20 ml of 1% sulfuric acid which the method normally employs to stop enzyme action, then add the extract and determine the ferricyanide reduction brought about by 5 ml of the mixture. The further precaution of using malt equivalents such that 40% conversion of starch to maltose is not exceeded was satisfied for malts up to about 240° Lintner by using for hydrolysis 2 ml of a 1-to-100 "free" extract or 1 ml of a 1-to-100 "total" extract.

Alpha dextrinogenic (alpha-amylase) activity: Alpha-amylase activity was determined by the method of Sandstedt, Kneen, and Blish (1939). In this method the rate of dextrinization in the presence of an excess of beta-amylase is measured and activity expressed in terms of *alpha-amylase units* (the number of grams of soluble starch which, under the influence of an excess of beta-amylase, are dextrinized by 1 gram of malt in 1 hour at 30°C).

Beta saccharogenic (beta-amylase) activity: From the malt saccharogenic and alpha dextrinogenic activities the beta-amylase activity

was calculated according to the technique of Kneen and Sandstedt (1941). In this technique the maltose production attributable to alpha-amylase alone is determined and subtracted from the maltose produced by the action of unmodified malt extract on soluble starch. The difference represents maltose production by the action of the beta component. Results are recorded as *beta-amylase units* (the number of grams of soluble starch converted to maltose by the beta-amylase of 1 gram of malt in 1 hour at 30°C).

Malt dextrinogenic activity: As used in this communication the term "malt dextrinogenic activity" signifies the ability of unmodified malt extract to dextrinize soluble starch. It was determined under conditions identical to those used for alpha dextrinogenic activity with the exception that the addition of an excess of beta-amylase was omitted. Accordingly, to 20 ml of 2% buffered Lintner starch were added water and extract sufficient to bring the total volume to 30 ml and dextrinization carried out at 30°C. Dextrinization time was recorded in minutes as the period elapsing between the start and the point at which the reaction mixture gave with iodine the "red-brown" color described by Sandstedt, Kneen, and Blish (1939). *Malt dextrinogenic units* were calculated as the number of grams of soluble starch dextrinized by 1 gram of malt in 1 hour at 30°C.

Starch-liquefying activity: Liquefying activity was determined by the method of Józsa and Gore (1930) as modified by Hollenbeck (1939). The modifications consisted of using glass beakers for starch pasting containers, a slightly different starch concentration (4.36 g dry starch per 100 g suspension), and a reaction temperature of 30°C. Liquefaction was measured over a 30-minute period. Percent decline in outflow time was calculated from the outflow times of unmodified starch, starch after 30 minutes of malt liquefaction, and completely liquefied starch. The actual weight of starch liquefied in 30 minutes was calculated from a preestablished relationship between decline in outflow time and grams of starch liquefied. The data were translated to conform with those of Józsa and Johnston (1935) and "liquefons" calculated from their published tables. *Starch-liquefying activity* was then expressed as "liquefons per gram of malt." The values as calculated represent a 1-hour run at 30°C and therefore are considerably higher than would be found at the 21°C reaction temperature used by Józsa and Johnston.

Activity on raw starch: The action of the malt extracts on raw (unboiled) starch was measured in a manner similar to the technique used by Blish, Sandstedt, and Mecham (1937). To 10 g of raw wheat starch were added 10 ml of a 1-to-20 malt extract and sufficient water and acetate buffer [5] (pH 4.7) to bring the total liquid volume to 100 ml. Toluol

[5] Buffer concentration was 3.0 ml glacial acetic acid and 4.1 g anhydrous sodium acetate per liter of reaction liquid.

was used as a preservative and hydrolysis carried out at 30°C. Aliquots for the determination of reducing substances by the ferricyanide method were taken after 4 and 24 hours of action. Reducing power was arbitrarily calculated as milligrams of maltose and the *activity on raw starch* expressed as milligrams of maltose produced by 1 g of malt in the 20-hour interval between the 4th and 24th hours of hydrolysis.

Autolytic diastatic activity: The method of Blish and Sandstedt (1933) was used to determine maltose production by the autolysis of the barley malt meals. To 5 g of meal were added 46 ml of acetate buffer (pH 4.7) and the mixture digested for one hour at 30°C. The reaction was stopped with sulfuric acid and sodium tungstate and reducing substances determined in the filtrate by the ferricyanide method. Using the method of Sandstedt (1939) for reducing sugars in flour, blank determinations were made on all the samples and these values subtracted from those obtained by autolysis. This resulted in an estimation of the actual amount of reducing substances produced. *Autolytic diastatic* activity was calculated as milligrams of maltose produced by the autolysis of 1 gram of malt for 1 hour at 30°C.

Results

All the data obtained on the twelve malts are presented in Table I. In this table the malts are arranged in the order of their Lintner values.[6]

TABLE I

STARCH-DEGRADING PROPERTIES OF "FREE" AND "TOTAL" BARLEY MALT EXTRACTS AND AUTOLYTIC DIASTATIC ACTIVITIES OF THE MEALS

Malt	Free extract							Autolytic diastatic activity	Total extract			
	Lintner value	Malt saccharogenic activity	Beta-amylase activity	Malt dextrinogenic activity	Alpha-amylase activity	Starch-liquefying activity	Activity on raw starch		Malt saccharogenic activity	Beta-amylase activity	Malt dextrinogenic activity	Alpha-amylase activity
	deg	*units*	*units*	*units*	*units*	*liquefons per g*	*mg maltose*	*mg maltose*	*units*	*units*	*units*	*units*
A	247	33.7	30.5	38.9	64.2	1227	1690	113	43.1	38.4	50.1	95.6
B	215	30.6	23.4	51.7	145.5	2876	2010	155	40.6	32.0	60.9	172.7
C	208	30.3	25.3	42.3	101.3	2060	1930	132	39.4	32.9	55.8	132.6
D	187	23.9	22.4	22.8	30.8	602	1360	82	35.7	33.5	30.0	44.0
E	168	23.6	20.2	34.6	66.1	1227	1760	126	27.4	22.8	40.6	92.7
F	154	20.7	18.5	26.7	43.5	787	1250	98	26.4	23.6	33.0	57.7
G	147	18.9	16.7	23.4	45.1	882	1280	94	25.9	22.9	33.2	61.4
H	133	18.0	14.5	28.8	69.4	1255	1800	121	22.3	17.6	35.6	92.3
I	116	15.1	12.3	22.7	55.8	1066	1480	97	18.6	15.1	28.7	69.6
J	113	14.8	13.0	18.0	36.9	655	1220	75	19.6	17.3	24.2	45.1
K	88	11.9	8.8	19.9	61.1	1227	1540	103	14.8	11.0	25.2	71.9
L	71	8.8	6.8	13.5	38.8	697	1200	76	15.9	13.4	22.9	49.6

[6] Lintner values were supplied by Dr. Allan D. Dickson of the Wisconsin Station.

Saccharogenic, dextrinogenic, beta-amylase, and alpha-amylase activities are given for both the " free " and " total " (papain) extracts. In addition starch-liquefying activity and activity on raw wheat starch are given for the free extracts and autolytic diastatic activities for the malt meals.

In order to minimize the possibility of enzymic changes during aging of the dry malts the data recorded in Table I were all obtained as rapidly as possible following the determination of the Lintner values. Much of the data was checked by two and in some cases three investigators, and it is regarded as possessing a high degree of accuracy. In view of the complexity of Table I various interrelations between the amylase activities will be discussed separately.

Discussion

Saccharogenic activity: Table II lists relative data comparing Lintner values and free saccharogenic and beta-amylase activities for the twelve malts. The data in each column were calculated on the basis of assign-

TABLE II

RELATIONSHIP BETWEEN LINTNER VALUES, MALT SACCHAROGENIC UNITS (KNEEN AND SANDSTEDT, 1941) AND THE ACTIVITY OF THE BETA-AMYLASE COMPONENT

Malt	Free extract		
	Lintner value	Malt saccharogenic activity	Beta-amylase activity
A	100	100	100
B	87	91	77
C	84	90	83
D	76	71	73
E	68	70	66
F	62	61	61
G	60	56	55
H	54	53	48
I	47	45	40
J	46	44	43
K	36	35	29
L	29	26	22

ing a value of 100 to sample A. It is obvious that there is a high degree of correlation between saccharogenic activity as determined by the method of Kneen and Sandstedt (1941) and as determined by the conventional " Lintner " method. While agreement is not absolute it is probably as close as might be expected when comparing data obtained by two different methods in two separate laboratories. Numerically the Lintner values average 7.5 times the saccharogenic units. Many cereal laboratories are not equipped for determining saccharogenic activity by the Lintner technique but are equipped for the determination of " Kneen-

Sandstedt units." Pending the accumulation of more data it appears that this factor of 7.5 may be used for converting saccharogenic units to degrees Lintner or *vice versa.*

The data of Table II show that there is good correlation between beta-amylase activity and malt-saccharogenic activity. This indicates that beta-amylase is the component principally responsible for malt saccharification. Actually the degree of saccharification due to beta-amylase alone ranges from 74.0% for malt K to 93.7% for malt D, with an average value of 84.1% for the twelve malts under consideration. These figures agree fairly well with the data of Hills and Bailey (1938), who estimated that from 25% to 29% of the saccharifying action of germinated barley was due to alpha-amylase. However, it should be emphasized that, in the comparison of two malts, higher saccharogenic activity does not necessarily mean higher beta-amylase content. For example the relatively high *alpha-amylase* contents of malts B and I result in their exhibiting a higher saccharogenic activity than would be predicted solely on the basis of their beta-amylase contents.

TABLE III

ALPHA-AMYLASE ACTIVITY AND ITS RELATION TO STARCH LIQUEFACTION, RAW STARCH DEGRADATION, AND AUTOLYTIC DIASTATIC ACTIVITY

Malt	Free extract				Autolytic diastatic activity
	Alpha dextrinogenic activity	Starch liquefying activity	Activity on raw starch	Malt saccharogenic activity	
B	100	100	100	100	100
C	70	72	96	99	85
H	48	44	90	59	78
E	45	43	88	77	81
A	44	43	84	110	73
K	42	43	77	39	66
I	38	37	74	49	63
G	31	31	64	62	61
F	30	27	62	68	63
L	27	24	60	29	49
J	25	23	61	48	48
D	21	21	68	78	53

Alpha-amylase activity: In Table III are listed relative data for alpha-amylase activities of the free extracts and for those actions which appear to be related to the alpha-amylase content of the malts. These include starch liquefaction, activity on raw starch, and autolytic diastatic activity. In addition values for malt saccharogenic activity are included for comparison. The data in each column were calculated on the basis of assigning a value of 100 to sample B and the samples listed in order of decreasing alpha-amylase activity.

From Table III it is apparent that there is excellent agreement between alpha dextrinogenic activity and the capacity of a malt to liquefy starch. It may be concluded that, in the ratio of malt to starch used, the beta-amylase content of the extract had no effect on the rate of liquefaction. Likewise the data indicate that the alpha-amylase activity of a malt extract may be measured equally well by both the dextrinization method of Sandstedt, Kneen, and Blish (1939) and the liquefaction technique of Józsa and Johnston (1935). Numerically the values for "liquefons per gram" average 19.0 times those for "alpha-amylase units," and it appears that this factor may be used for interconversion between the two types of alpha-amylase values when both are determined at 30°C. However, the liquefying and dextrinizing actions of alpha-amylase should not be confused. Liquefaction by alpha-amylase is characterized by inappreciable production of dextrins and may be just a preliminary step before the eventual dextrinization of the starch takes place (Hollenbeck and Blish, 1941). In addition, the "amylophosphatase" conception of Waldschmidt-Leitz, Samec, and Mayer cannot be ignored. As summarized by Mayer (1939) the findings of these authors indicate that enzymic starch liquefaction can take place without the production of either dextrins or reducing sugars. The present status of the problem suggests that starch liquefaction as measured by the method of Józsa and Johnston (1935) be designated as "alpha liquefying activity" and starch dextrinization in the presence of an excess of beta-amylase (Sandstedt, Kneen, and Blish, 1939) as "alpha dextrinogenic activity."

The data of Table III show that the ability of malt to hydrolyze raw wheat starch is highly correlated with alpha-amylase activity. The small spread between the actions of those malts of highest alpha-amylase activity resulted in large part from the ratio of malt extract to raw starch. When half concentrations of malts B and C were used on the regular amount of starch the relative values for these two samples were respectively 100 and 88. The raw-starch activity of sample B shows lack of correlation with alpha-amylase activity. Upon further investigation this extract was found to be abnormally high in maltase activity. The amount of glucose produced from preformed maltose during a 24-hour hydrolysis may be appreciable. As pointed out by Blish, Sandstedt, and Kneen (1938) when all ferricyanide reducing substances are calculated as maltose, as in the measurement of activity on raw starch, the *apparent* maltose production is higher than actually occurs and malt activity is incorrectly evaluated.

The close relationship between alpha-amylase activity and activity on raw starch also suggests the strong possibility that the "raw starch factor" of Blish, Sandstedt, and Mecham (1937) may be alpha-amylase.

"Alpha-amylase values" as reported by these authors and by Blish, Sandstedt, and Kneen (1938) were determined by the unmodified Wohlgemuth technique (dextrinization by the combined action of alpha- and beta-amylase) and as such do not represent a measure of the actual alpha-amylase activity. Conclusions which these authors based on comparisons between "alpha-amylase activity" and activity on raw starch are therefore of questionable value. The present data support the hypothesis of Brown and Morris (1890), who concluded that the amylase responsible for disintegrating raw starch was the "liquefying" enzyme which appears when grain is germinated.

The autolytic diastatic activity of malt was found by Sallans and Anderson (1939) to be correlated with liquefying activity of the extract. The work of these authors also confirmed the finding of Shellenberger and Bailey (1936) that autolytic activity was not correlated with the saccharifying power of the malt extract. The results shown in Table III likewise indicate a fairly good correlation of autolytic diastatic activity with alpha-amylase content and a lack of correlation with malt saccharogenic activity. The data therefore confirm the conclusion that alpha-amylase content is a major factor governing the rate of autolytic maltose production by malt meals. Sallans and Anderson (1939) also found a significant correlation between malt-saccharifying and malt-liquefying activities. The data of Table III are not in agreement [7] with this finding and indicate that the alpha-amylase activity of a malt can *not* be predicted on the basis of the saccharogenic activity of the extract.

The fact that saccharogenic activity is of little value in estimating the alpha-amylase activity of malt assumes considerable importance in the cereal industry. When malt is used as a flour supplement it is being introduced into a medium already well supplied with beta-amylase. Under these conditions the alpha component of malt amylase must be considered as having major significance. Maximum influence on the flour would be expected from malts of high alpha-amylase content, not necessarily from those of high saccharogenic power.

Malt dextrinogenic activity: That beta- and alpha-amylase are both operative in the rate at which the action of malt causes starch to lose its ability to give the typical starch-iodine color reaction has been recognized by Blom, Bak, and Braae (1937) and Hanes and Cattle (1938). Sandstedt, Kneen, and Blish (1939) found that when an excess of beta-amylase was present, dextrinization of soluble starch proceeded nearly six times as fast as when the same amount of alpha-amylase acted alone. Further studies indicate that this ratio is slightly too high. The average value for numerous determinations was found to be 5.4; that is, in the

[7] The correlation coefficient between malt-saccharifying and malt-liquefying activities was found to be + .6026.

presence of an excess of beta-amylase the rate of dextrinization of soluble starch is 5.4 times as rapid as would be caused by the equivalent amount of alpha-amylase acting independently on such starch. A "true" measure of alpha-dextrinogenic activity would therefore be attained by dividing the alpha-amylase "units" of Sandstedt, Kneen, and Blish (1939) by 5.4.

Table IV shows the manner in which the alpha- and beta-amylase activities of the twelve malts are related to the dextrinogenic activities. Data are listed for beta-amylase activity (A), alpha-amylase activity divided by 5.4 (B), the summation of these two activities (A + B), and for malt dextrinogenic activity. With the possible exception of sample D there is rough numerical agreement between the unit values determined for malt-dextrinogenic activity and the values derived from the summa-

TABLE IV

THE RELATIONSHIP OF ALPHA- AND BETA-AMYLASE CONTENTS OF MALT TO STARCH DEXTRINIZATION

Malt	A Beta-amylase activity	B Alpha-amylase activity	A+B	Malt dextrinogenic activity
	units	*units/5.4*		*units*
A	30.5	11.9	42.4	38.9
B	23.4	27.0	50.4	51.7
C	25.3	18.8	44.1	42.3
D	22.4	5.7	28.1	22.8
E	20.2	12.2	32.4	34.6
F	18.5	8.1	26.6	26.7
G	16.7	8.4	25.1	23.4
H	14.5	12.9	27.4	28.8
I	12.3	10.3	22.6	22.7
J	13.0	6.8	19.8	18.0
K	8.8	11.3	20.1	19.9
L	6.8	7.2	14.0	13.5

tion of the beta-saccharogenic and "true" alpha-dextrinogenic units. Further investigation must be carried out before any final conclusion may be drawn regarding this possible additive nature of alpha- and beta-amylase in dextrinization. However, it does appear that an approximate evaluation of the *beta*-saccharogenic activity of a malt may be obtained simply by subtracting alpha-amylase units (divided by 5.4) from malt-dextrinogenic units.

Since the action of beta-amylase alone does not appreciably alter the color which starch gives with iodine, it would seem that the chief function of this component in dextrinization is to degrade the starch to alpha-amylodextrin. A further function is indicated by the fact that the presence of beta-amylase likewise increases the rate at which alpha-amylodextrin is dextrinized by alpha-amylase (Sandstedt, Kneen, and Blish,

1939). The role of beta-amylase in dextrinization then becomes the degradation of starch to alpha-amylodextrin followed by the hydrolysis of some of the products resulting from the action of alpha-amylase on this dextrin.

"Latent" amylase: The latent amylase of germinated or ungerminated barley may be designated as that fraction which requires the action of a proteolytic enzyme such as papain to render it extractable by water. In Table V data are listed showing the amounts of latent amylase and the percentages of total amylase in the latent condition for both the alpha- and beta-components.

The data of Table V show that, as with the free amylases, there is wide variation in the amounts of latent beta- and alpha-amylase in malts. With the exception of one or two individual samples a fairly constant percentage of the total amylase exists in the latent state. The amounts

TABLE V

THE LATENT ALPHA- AND BETA-AMYLASE OF MALTS

Malt	Beta-amylase activity		Alpha-amylase activity	
	Latent	Relation of latent to total	Latent	Relation of latent to total
	units	%	*units*	%
A	7.9	20.6	31.4	32.9
B	8.6	26.9	27.2	15.8
C	7.6	23.1	31.3	23.6
D	11.1	33.1	13.2	30.0
E	2.6	11.4	26.6	28.7
F	5.1	21.6	14.2	24.6
G	6.2	27.1	16.3	26.6
H	3.1	17.6	22.9	24.8
I	2.8	18.5	13.8	19.8
J	4.3	24.9	8.2	18.2
K	2.2	20.0	10.8	15.0
L	6.6	49.3	10.8	21.8
Average		24.5		23.5

varied from 11.4% to 49.3% for the beta component and from 15.0% to 32.9% for the alpha component. The average percentages of latent amylase were about the same for the two components: 24.5% of the beta-amylase and 23.5% of the alpha-amylase. This agreement does not hold for individual malts. For example 49.3% of the beta-amylase was latent in sample L and only 21.8% of the alpha component.

The necessity for including determinations of "total" amylase in malt-analysis programs has not been established. Certainly in instances where prolonged action of malt on starch substrate is permitted some attention should be paid to the amylase liberated by proteolytic activity.

Summary and Conclusions

Values for malt saccharogenic activity, beta-amylase activity, malt dextrinogenic activity, and alpha-amylase activity were determined for both the " free " and " total " (papain) extracts from twelve barley malts. In addition starch-liquefying activity and " activity on raw starch " were determined for the " free " extracts and autolytic diastatic activity for the malt meals.

The data presented indicate that no one of the methods commonly used for determining the starch-degrading property of malt can be expected to provide a complete picture of the amylolytic potentialities. Individual determinations of saccharification, dextrinization, or liquefaction of starch measure accurately only the particular function investigated. Generally, malts of high saccharogenic activity have high dextrinogenic activity. However no prediction of relative liquefying power may be made from either of these activities.

Accurate estimation of the amounts of the individual alpha- and beta-amylases in malt is possible only by the use of methods designed specifically for the purpose. While saccharogenic values are largely dependent on *beta-amylase* and therefore give some idea as to relative content of this component, *alpha-amylase* may be estimated only by the use of methods in which varying content of beta-amylase does not influence the results. Present evidence indicates that either the starch-liquefaction method of Józsa and Johnston (1935) or the modified Wohlgemuth technique of Sandstedt, Kneen, and Blish (1939) is specific for the alpha component. In view of the present uncertainty regarding the exact relationship between starch liquefaction and starch dextrinization it is suggested that the liquefying power of alpha-amylase be termed " alpha liquefying activity " and the dextrinizing power of this component termed " alpha dextrinogenic activity."

It appears that the two most significant of the amylolytic determinations commonly made on malt are the *malt saccharogenic activity* and the *alpha dextrinogenic activity*. From these two determinations the beta-amylase activity may be calculated. The summation of the alpha- and beta-amylase activities provides an approximation of the malt dextrinogenic activity. Alpha-amylase values alone accurately measure starch-liquefying power, provide an estimation of the ability of the malt extract to hydrolyze raw wheat starch, and permit conclusions as to the probable autolytic diastatic activity of the malt meal itself. It would seem then that any description of the amylolytic power of a malt must include at least values for these two activities: malt saccharogenic activity and alpha-amylase activity.

Two conversion factors were calculated and are tentatively proposed:

Malt saccharogenic units (Kneen and Sandstedt, 1941) $\times$ 7.5 = degrees Lintner.

Alpha dextrinogenic units (Sandstedt, Kneen, and Blish, 1939), $\times$ 19.0 = starch-liquefying activity in liquefons per gram (when the reaction temperature is 30°C for both).

Fairly good agreement was found between the amylolytic activities of the total extracts and the corresponding activities of the free extracts. In general then, malts high in " free " amylase are usually high in " total " amylase. However individual variation makes any attempt at accurate prediction unwarranted.

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PARALLELISM BETWEEN STARCH DEXTRINIZING AND LIQUEFYING ACTIVITIES OF AMYLASES [1, 2]

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Three types of activities have been noted when boiled or gelatinized starch is treated with diastatically active enzymes such as are found in malt and other biologically active substances: (1) saccharification, characterized by a progressive development of reducing sugars, chiefly maltose and glucose, (2) dextrinization, characterized by the conversion of the starch to dextrins, which is denoted by a loss of the starch's characteristic property of turning blue when treated with iodine, and (3) liquefaction, indicated by a rapid decrease in the viscosity of the starch paste.

Although little is known of what takes place chemically when starch paste is liquefied by malt, it is not unreasonable to suppose that this liquefaction, like saccharification and dextrinization, is also a manifestation of the hydrolytic cleavage of the starch molecule. Thus, either the action of the dextrinizing component of malt amylase, *alpha-amylase*, or the saccharifying component, *beta-amylase*, or of both acting together, might reasonably be expected to contribute to the liquefying process Some investigators believe, however, that this liquefaction is independent of both dextrinization and saccharification and the possibility of an independent *liquefying component* has been pointed out. Chrzaszcz and Janicki (1932) present a summary of the work pertinent to this question up to the year 1932.

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[2] Published with the approval of the Director as paper No. 294, Journal Series, Nebraska Agricultural Experiment Station.
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Later Waldschmidt-Leitz and Mayer (1935) reported the isolation of a distinct liquefying amylase from barley malt. These authors designated this component as an *amylophosphatase,* and attributed its liquefying activity to a disaggregation caused by the splitting off of esterified phosphorus from the starch aggregates. Friedmann (1939) disagreed with these authors and found that phosphorus was not liberated upon liquefaction. Mayer (1939) showed that starch which has been treated with a low concentration of iodine is resistant to saccharification but is still liquefied by an amylase preparation. Lüers and Rümmler (1935) claimed that the destruction of the saccharogenic amylase by heating an extract of malt to 70°C for 15 minutes had little effect on liquefying power, but decreased dextrinization markedly. Samec and Dolar (1939) showed that the initial change of liquefaction of a starch paste is independent of the change in reducing power or iodine color. These changes were thus supposedly related to different partial processes in the degradation of the starch molecule. On the other hand Józsa and Johnston (1935) and Blom, Bak, and Braae (1937) believe that liquefaction is chiefly attributable to the activity of alpha-amylase, and that this function can be measured quantitatively by viscometric procedure. Friedmann (1939) reported that *both* the dextrinizing and saccharifying amylases contribute to the liquefying process.

The various methods for determining the nature and degree of starch degradation by diastases have as their basis one or more of these three types of activities. Examples of the three different types of methods for following the amylolytic degradation of starch are: (1) the well known Lintner method and the Blish and Sandstedt (1933) method [4] for the saccharification of starch, (2) the Wohlgemuth (1908) and the Sandstedt, Kneen, and Blish (1939) methods for the dextrinization of starch, and (3) the Józsa and Gore (1930) and the Blom and Bak (1938) methods for the liquefaction of starch.

In the interests of improved technological control in the cereal industries, with special reference to baking, to malting, and to other industrial activities involving starch degradation, there is a recognized need for more certain knowledge as to the relative selectivities and values of the various types of methods available for measuring amylolytic processes and for interpreting their manifestations. Is it indeed true that the liquefaction and dextrinization of starch pastes are both *attributable* to the action of a single enzyme, *alpha-amylase?* If this is so, which of the two types of procedure suitable for the estimation of alpha-amylase activity, *i.e.,* the Wohlgemuth type of method or the viscometric method, is preferable and why?

[4] Modified to measure maltose production using an amylolytic active extract and a starch paste substrate.

The present report deals with studies undertaken in an attempt to obtain further information about the foregoing and related matters. The principal method of approach in these studies has been to note the presence or absence of a *parallelism* between liquefying and dextrinizing properties, using amylase preparations from different sources. This approach, which is similar to that adopted by Blom, Bak, and Braae (1938), is based upon the supposition that if the two functions are affected to an equal degree by alterations in temperature, pH, salt additions, etc., it is highly probable that only one enzyme is the factor involved. Lack of such parallelism in these respects would presumably indicate that more than one biocatalyst enter into the process.

Amylases were selected from three widely different sources: malted wheat, *Aspergillus oryzae* (takadiastase, commercial powder, Parke Davis Co., Detroit), and a bacterial preparation (commercial powder, Wallerstein Co., New York).

Methods

Liquefying activity: The viscometric method as proposed by Józsa and Gore (1930) was used for the determination of the liquefying activities of the amylases. This method was selected mainly because of its simplicity and adequacy for the purpose of this investigation.

A 100-ml pipette was used as the viscometer. This pipette was surrounded by a water jacket through which water at 30°C was circulated. The delivery tube of the pipette was marked 6 cm below the bulb and the time of flow from mark to mark of an 87.5% glycerol-water solution (sp gr 1.2213 at 30°) was 161 to 162 seconds at 30°C. The outflow time for water at that temperature was 36 to 37 seconds. The pipette was attached to an aspirator in such a manner that it could be filled or emptied at will by turning a two-way stopcock in the line between the pipette and aspirator.

The method of preparation of the standard starch paste was essentially the same as that used by Józsa and Gore (1930). A 4.36% (moisture-free basis) potato-starch paste was used but a detailed description of its preparation is omitted here since the modifications from the method cited were merely adaptations of the type of pasting containers, the high-speed stirrer, and the pipette viscometer used. The method cited was also modified to the extent that all the reactions were run and viscosity measurements were made at 30°C instead of 21°C. The standard paste flowed from the pipette viscometer at this temperature in 165 seconds with a deviation of 5 seconds between different preparations.

Dextrinizing activity: The Wohlgemuth procedure was used for the determination of the dextrinizing activities. The dextrinizing activity of an amylase preparation such as malt, which contains both alpha- and

beta-amylase, is a combined function of both types of amylases. In the following report a distinction is made between dextrinization and *alpha dextrinization* to the extent that the latter term is applied to the dextrinization due solely to the alpha-amylase of malt or of any other amylase preparation in which both types of enzymes are present.

Alpha dextrinizing activity: The method of Sandstedt, Kneen, and Blish (1939) was used. In this method an excess of beta-amylase is added to eliminate the effect of this factor in the dextrinization process. With this excess of beta-amylase present, the rate of production of starch degradation products, which give the standard iodine coloration, is dependent only on the activity of the alpha-amylase present in the enzyme preparation added.

Experimental

Determination of a liquefying curve: A curve showing the effect of liquefaction upon the outflow time of the paste was determined. Several lots of starch paste were prepared and different amounts of unliquefied and fully liquefied paste were mixed and the outflow time recorded. The fully liquefied starch was obtained by adding 15 ml of a concentrated malt extract to 150 g of the paste, allowing it to act for 30 minutes, and then boiling the paste for 3 minutes to inactivate the enzyme.

The results are shown in Figure 1. Each point on the curve represents an average of several determinations.

Effect of malt concentration on liquefaction: A typical curve showing the effect of various amounts of an amylase preparation upon the amount of starch liquefied is shown in Figure 2. The extracts from the various amounts of malted wheat flour were added to 150 g of the standard starch paste and the viscosity measured at the end of one hour. The amount of starch liquefied was then determined by referring to the curve in Figure 1.

Determination of the effect of beta-amylase upon liquefaction: An effort was made to determine the extent to which beta-amylase affected the liquefying process observed with malt. The liquefying activity of a flour extract (ungerminated soft wheat), which supposedly contained little or no alpha-amylase, was determined. It was observed that compared to malt wheat flour, over 160 times by weight as much unmalted flour was necessary to give the same amount of liquefaction. The wheat-flour extract showed a very high beta-amylase activity (measured by the rate of conversion of a starch paste to maltose by the Blish and Sandstedt ferricyanide method). The alpha-amylase activity of this extract was found to be very slight since it failed to give a detectable change in blue iodine coloration between a 1-hour and a 24-hour period of action upon a starch paste.

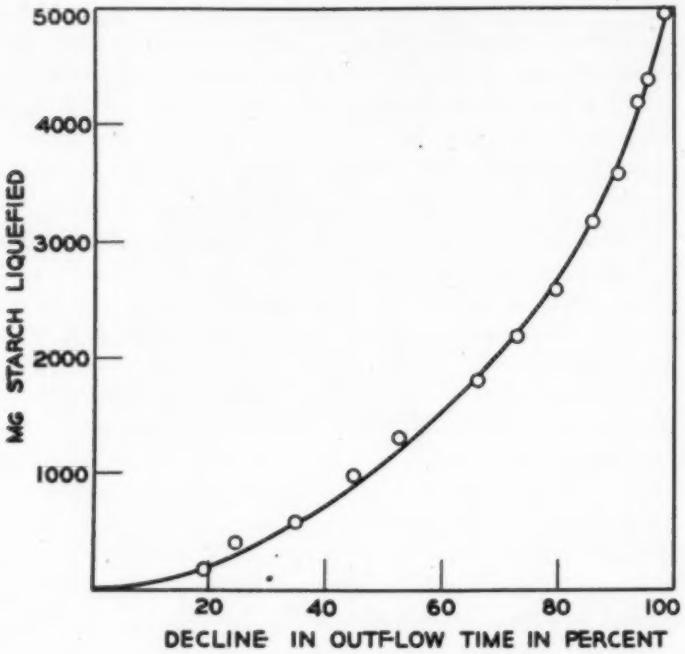

Fig. 1. Decline in outflow time with amount of starch liquefied.

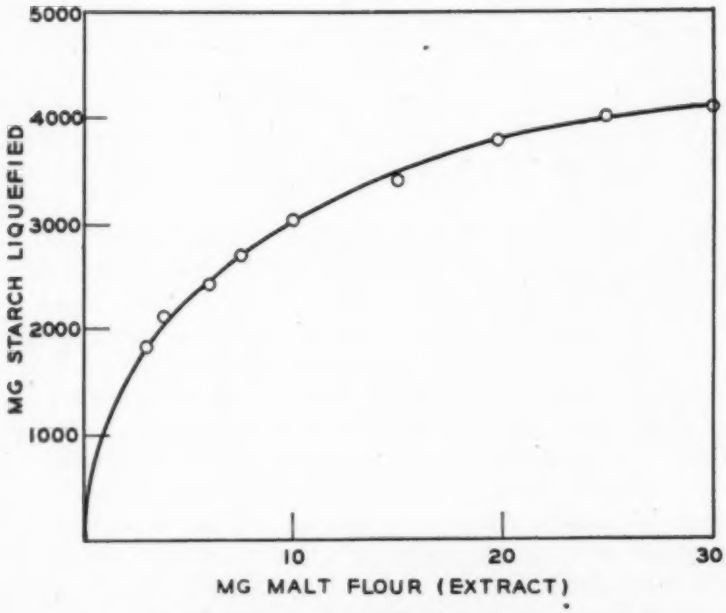

Fig. 2. Amount of starch liquefied with various amounts of malt flour.

It is shown by the data of Table I that the beta-amylase present in the small quantity of malt used for rapid liquefaction of the paste has a negligible effect in the liquefying activity of the malt. A malt extract was heated after the method of Ohlsson (1930) (70°C for 15 minutes) to inactivate the beta-amylase present. The percent of the original alpha-amylase which remained after this treatment was then determined. It was found that 84% of the original alpha-amylase activity remained. From this it was calculated that 1.2 ml of the heated preparation contained the same amount of alpha-amylase as 1.0 ml of the unheated, but the unheated contained a large amount of beta-amylase compared to the heated. The liquefying activities of these preparations were then compared in this ratio: 1 of the unheated to 1.2 of the heated. Closely corresponding outflow times would indicate that the beta-amylase present in the small amount of malt used did not affect the liquefaction.

TABLE I

COMPARISON OF THE LIQUEFYING POWERS OF HEATED AND UNHEATED MALT EXTRACTS, WHICH CONTAIN THE SAME AMOUNT OF ALPHA-AMYLASE

Malt extract concentration in 1,000 ml solution		Outflow time	
Unheated malt	Heated malt	Unheated malt	Heated malt
ml	*ml*	*sec*	*sec*
1.0	1.2	78.8	78.2
1.2	1.5	72.2*	73.2
2.0	2.4	65.0	63.8
5.0	6.0	50.8	50.8
10.0	12.0	42.6	44.6

Determination of the extent of dextrinization occurring during liquefaction: An indication of the amount of dextrinization occurring during liquefaction can be obtained by comparing the time necessary for a liquefied or partially liquefied starch substrate to show the loss of blue iodine coloration, when acted upon by alpha-amylase, with the corresponding time for a nonliquefied portion.

Several 150-g samples of the standard starch paste were treated with 15 ml of a malt solution containing the extract of 40 mg of malt. At selected time intervals 10-ml portions of these samples were taken out and added to 10 ml of hot water and the mixture placed in a boiling water bath for 10 minutes. After cooling to 30°C each portion was treated with 10 ml of an alpha-amylase solution and the reaction time necessary to reach the standard iodine end-point was determined. The data given in Table II suggest that the amount of dextrinization occurring during the process of liquefaction is quite small.

Assuming 20 minutes to be necessary for the complete dextrinization

TABLE II

DETERMINATION OF THE EXTENT OF DEXTRINIZATION OCCURRING DURING VARIOUS STAGES OF THE LIQUEFACTION PROCESS

Time intervals	Time of outflow of paste	Decline in outflow time	Dextrinizing time	Approximate amount of dextrinization
min	*sec*	%	*min*	%
0	167.0	0.0	20.0	0.0
15	50.4	89.7	19.5	2.5
30	43.8	94.8	19.5	2.5
45	41.6	96.7	19.5	2.5
60	40.6	97.3	19.0	5.0
120	38.0	99.4	19.0	5.0

of the 10-ml aliquots, it was found that only about 5% or less of the dextrinization occurred during the liquefaction. This would indicate that the liquefaction is largely a preliminary step which occurs before very much of the starch is actually converted into dextrins. This conclusion agrees with that of Samec and Dolar (1939).

Comparison of the liquefying powers of different amylase preparations on the basis of equal dextrinizing activities: The concentrations of solutions of takadiastase, bacterial amylase, and malt alpha-amylase (heated malt extract) were adjusted until they showed equal dextrinizing activities as measured by the Wohlgemuth method. Using equivalent concentrations of these solutions, the liquefying powers were determined in order to note whether the adjustment to equal dextrinizing powers had also equalized the liquefying functions.

According to the preliminary determinations with the Wohlgemuth method it was found that the heated extract of 100 mg of the malt, 0.82 mg of the bacterial amylase preparation, and 3.0 mg of the takadiastase had equivalent dextrinizing activities. These amounts were dissolved in 100 ml of water and 15-ml portions of these solutions (150 g of starch paste) were used in the determination of the liquefying activity. The times of outflow of the starch paste at the end of 30 minutes were as follows: takadiastase 70.4 seconds, bacterial amylase 63.0 seconds, and alpha-amylase 64.0 seconds. If the dextrinizing and liquefying activities are functions of a single enzyme, the outflow times of the pastes would have been a constant value since the liquefying activities would have been equivalent.

The possibility arises that perhaps a small amount of a beta-amylase type component in the takadiastase accounted for the lack of parallelism between the two activities in the case of this preparation. If the takadiastase contained an amylase similar to beta-amylase along with its alpha-amylase type, this enzyme would probably increase the dextrinizing activity as measured by the Wohlgemuth method, and consequently the

liquefying value of the amount showing the same dextrinizing activity as the other two enzymes would be lower. It is noted that the viscosity of the paste liquefied by the takadiastase is higher (liquefying activity of the amylase lower) than in the cases of the other two enzymes. This fact was further substantiated by a comparison of the two types of activities as in the preceding experiment but unheated malt extract, known to contain active beta-amylase, was substituted for the heated preparation (alpha-amylase). The liquefying activity of the malt showed a decided deviation from that of the other two preparations, since the times of outflow of the starch were 85.0 seconds for the malt, 70.4 seconds for the takadiastase, and 63.0 seconds for the bacterial amylase.

Better correlation between the two types of activities was obtained by eliminating the beta-amylase factor in dextrinization by the use of alpha dextrinizing values in place of dextrinizing values. For example it was found that the outflow times of the starch liquefied by equivalent alpha dextrinizing concentrations were: takadiastase 48.2 seconds, malt 46.0 seconds, and bacterial amylase 44.8 seconds.

Allowing for all the errors encountered in the two methods, it can be concluded that for solutions showing equal alpha dextrinizing activities, a reasonably close agreement exists between their liquefying values.

Parallelism in the effects of temperature changes on liquefying and dextrinizing activities: As boiling temperature is approached, amylase activity is rapidly destroyed. If dextrinization and liquefaction are both functions of the same enzyme, the destructive effect of heat on one should parallel the effect upon the other. This type of parallelism was investigated for all three of the amylase preparations. These studies were made both with and without the presence of $CaCl_2$ and NaCl, in view of the fact that the calcium ion is known to greatly increase the resistance of amylases to heat inactivation (Wallerstein, 1908; Nakamura, 1931).

Bacterial Amylase

Solutions of the bacterial amylase were heated to different temperatures and the extent of inactivation measured by the viscometric and the Wohlgemuth-type methods. Each of three 50-mg samples of the bacterial amylase powder was dissolved in 200 ml of water, 200 ml of 0.05*N* NaCl solution, and 200 ml of 0.05*N* $CaCl_2$ solution, respectively. Three 50-ml samples of each of these solutions were heated at 50°, 60°, and 70°C, respectively, for 15 minutes.

The dextrinizing values were determined by using the equivalent of 2.5 mg of the amylase (heated and unheated) to 20 ml of 2.5% potato starch (wet basis). The percent of activity remaining after these treatments can be calculated from these dextrinization values. The liquefying values were determined by using the equivalent of 0.1875 mg of the

amylase to 150 g of the starch paste. The viscosities were measured at the end of 30 minutes. The percent of liquefying activity remaining after the heat treatment can be calculated by means of the curve in Figure 3. The results are shown in Table III.

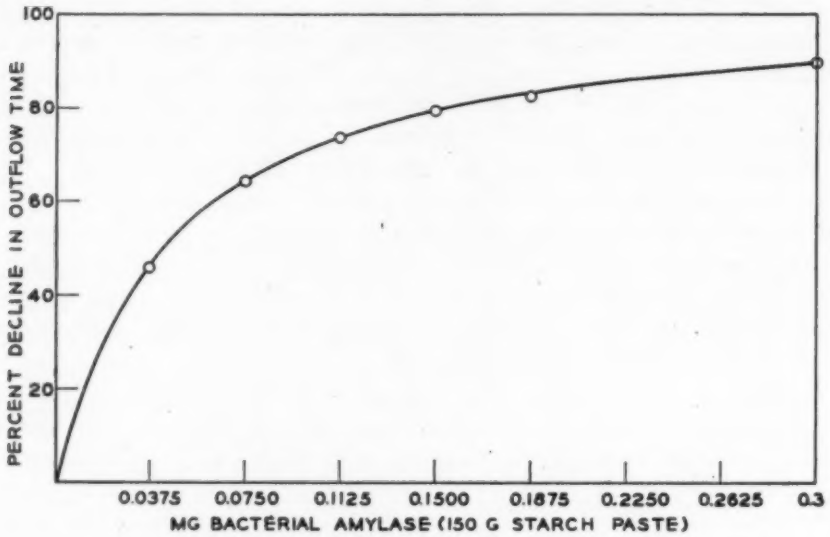

Fig. 3. Decline in outflow time of the starch paste when liquefied by various amounts of bacterial amylase.

TABLE III

DETERMINATION OF THE DEXTRINIZING AND LIQUEFYING ACTIVITIES REMAINING AFTER PARTIAL HEAT-INACTIVATION OF THE BACTERIAL AMYLASE PREPARATION

Heat treatment (temperature)	Solution	Dextrinizing time	Outflow time	Dextrinizing activity remaining	Liquefying activity remaining
°C		*min*	*sec*	%	%
Unheated	H_2O	16.0	59.0	100.0	100.0
Unheated	NaCl	16.0	59.4	100.0	100.0
Unheated	$CaCl_2$	16.0	59.6	100.0	100.0
50	H_2O	18.5	61.0	86.5	90.0
50	NaCl	18.5	61.5	86.5	90.0
50	$CaCl_2$	18.5	60.8	86.5	90.0
60	H_2O	26.0	67.0	61.5	70.0
60	NaCl	29.0	71.0	55.0	60.0
60	$CaCl_2$	22.5	63.5	71.0	80.0
70	H_2O	120.0	114.4	13.3	15.0
70	NaCl	197.0	124.4	8.0	11.0
70	$CaCl_2$	40.5	78.0	39.5	47.0
75	$CaCl_2$	137.0	121.4	11.5	12.0

The correlation between the values in the last two columns in Table III would indicate that approximately the same degree of inactivation by heat is shown in the liquefying activity as in the dextrinizing activity. The calcium ion seems to serve as a protective agent to the same extent for both the liquefying and dextrinizing functions.

Takadiastase

The same procedure was followed with the takadiastase as with the bacterial amylase. Solutions containing 1 mg per milliliter of the powdered takadiastase were heated to the specified temperatures. The dextrinizing activity was determined by the use of 10 mg of the amylase preparations to 20 ml of the 2.5% starch. The liquefying activity was determined with 1.5 mg of the amylase to 150 g of the standard starch paste, and the reaction was allowed to run for 30 minutes. The data are given in Table IV. The percent of liquefying activity remaining after heating was calculated from the curve in Figure 4.

TABLE IV

DETERMINATION OF THE DEXTRINIZING AND LIQUEFYING ACTIVITIES REMAINING AFTER PARTIAL HEAT-INACTIVATION OF THE TAKADIASTASE PREPARATION

Heat treatment (temperature)	Solution	Dextrinizing time	Outflow time	Dextrinizing activity remaining	Liquefying activity remaining
°C		*min*	*sec*	%	%
Unheated	H_2O	15.0	51.6	100.0	100.0
Unheated	NaCl	15.0	52.0	100.0	100.0
Unheated	$CaCl_2$	15.0	51.0	100.0	100.0
50	H_2O	19.0	56.2	79.0	68.0
50	NaCl	40.5	65.6	37.0	42.5
50	$CaCl_2$	15.0	51.4	100.0	100.0
60	H_2O	—	152.5	—	1.0
60	NaCl	—	158.4	—	1.0
60	$CaCl_2$	19.5	54.2	77.0	82.0
70	$CaCl_2$	—	148.6	—	1.0

The same tendency towards parallelism between the two activities was found for takadiastase as was demonstrated for bacterial amylase. The calcium ion again served as a protective factor against the heat inactivation to the same degree in both activities.

Malt Alpha-Amylase

Following the same procedure the two activities were compared by the use of malt alpha-amylase. Solutions containing alpha-amylase corre-

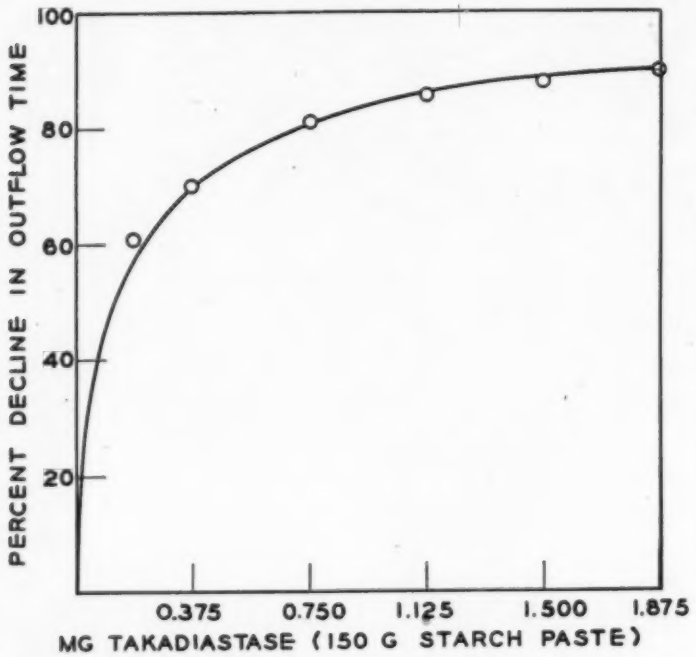

Fig. 4. Decline in outflow time of the starch paste when liquefied by various amounts of takadiastase.

sponding to the extract of 40 mg of the original malted wheat flour per milliliter were heated to the specified temperatures. Of these solutions the amounts corresponding to 400 mg and 30 mg of malt were used for the determinations of the dextrinizing and liquefying activities, respectively. A continued close agreement existed between the two activities as evidenced by the data given in Table V. The percent of liquefying activity remaining in each preparation after the heat treatments was determined by the use of Figure 5.

Malt Extract

To recheck the belief that beta-amylase in malt affects the dextrinizing power but not the liquefying power of the malt extract in the concentrations used for each method, partial heat inactivation was carried out with and without the use of an excess amount of beta-amylase in the determination of the dextrinization values.

Concentrations of the malt extract equivalent to those used for the malt alpha-amylase in the preceding determination were used here. More dilute solutions of the malt extract were heated and used for the determinations of the alpha dextrinizing activity, since presence of the

excess of beta-amylase decreased the Wohlgemuth times about 80% for the same amount of malt.

TABLE V

DETERMINATION OF THE DEXTRINIZING AND LIQUEFYING ACTIVITIES REMAINING AFTER PARTIAL HEAT-INACTIVATION OF THE MALT ALPHA-AMYLASE PREPARATION

Heat treatment (temperature)	Solution	Dextrinizing time	Outflow time	Dextrinizing activity remaining	Liquefying activity remaining
°C		*min*	*sec*	%	%
Unheated	H_2O	11.5	53.0	100.0	100.0
Unheated	NaCl	11.5	53.2	100.0	100.0
Unheated	$CaCl_2$	11.5	53.0	100.0	100.0
50	H_2O	13.0	54.0	88.5	90.0
50	NaCl	13.5	55.2	85.0	80.0
50	$CaCl_2$	12.5	53.8	92.0	91.0
60	H_2O	16.0	57.2	72.0	66.0
60	NaCl	18.0	60.2	64.0	56.0
60	$CaCl_2$	13.5	55.0	85.0	82.0
70	H_2O	—	120.5	—	5.0
70	NaCl	—	129.2	—	4.0
70	$CaCl_2$	23.5	63.0	49.0	50.0
75	$CaCl_2$	—	150.8	—	—

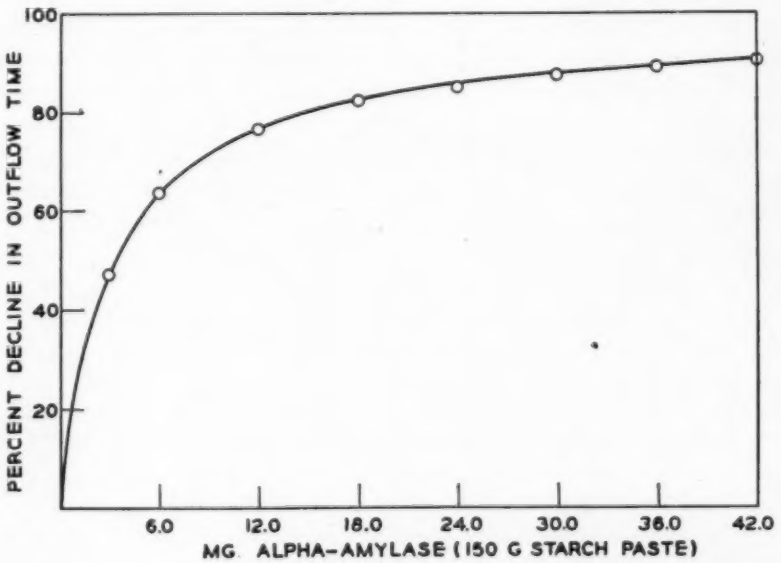

Fig. 5. Decline in outflow time of the starch paste when liquefied by various amounts of malt alpha-amylase.

The data obtained in the two instances are given in Tables VI and VII. The percent of liquefaction in each case is calculated by means of the curve in Figure 6.

The values for percent of activity as measured by the two methods show a much higher correlation in Table VII than in Table VI. Heating affected the amount of active beta-amylase present as well as the amount of alpha-amylase that remained active. With the low enzyme concentra-

TABLE VI

DETERMINATION OF THE DEXTRINIZING AND LIQUEFYING ACTIVITIES REMAINING AFTER PARTIAL HEAT-INACTIVATION OF THE MALT EXTRACT

Heat treatment (temperature)	Solution	Dextrinizing time	Outflow time	Dextrinizing activity remaining	Liquefying activity remaining
°C		*min*	*sec*	%	%
Unheated	H_2O	9.5	50.2	100.0	100.0
Unheated	NaCl	9.5	50.6	100.0	100.0
Unheated	$CaCl_2$	9.5	50.2	100.0	100.0
50	H_2O	10.0	50.6	95.0	100.0
50	NaCl	11.0	51.8	86.0	90.0
50	$CaCl_2$	10.0	50.6	95.0	100.0
60	H_2O	16.5	53.4	57.5	80.0
60	NaCl	34.0	58.0	28.0	60.0
60	$CaCl_2$	20.5	50.8	46.0	100.0
70	H_2O	197.0	91.0	4.8	2.0
70	NaCl	—	150.4	—	—
70	$CaCl_2$	27.5	53.8	34.5	78.0

TABLE VII

DETERMINATION OF THE ALPHA-DEXTRINIZING AND LIQUEFYING ACTIVITIES REMAINING AFTER PARTIAL HEAT-INACTIVATION OF THE MALT EXTRACT

Heat treatment (temperature)	Solution	Alpha dextrinizing time	Outflow time	Dextrinizing activity remaining	Liquefying activity remaining
°C		*min*	*sec*	%	%
Unheated	H_2O	18.5	50.2	100.0	100.0
Unheated	NaCl	18.5	50.2	100.0	100.0
Unheated	$CaCl_2$	18.5	50.4	100.0	100.0
50	H_2O	21.0	50.6	88.0	97.0
50	NaCl	28.5	54.6	65.0	68.0
50	$CaCl_2$	21.0	51.0	88.0	94.0
60	H_2O	31.0	57.6	60.0	61.0
60	NaCl	71.5	73.6	26.0	36.0
60	$CaCl_2$	22.0	51.4	84.0	91.0
70	H_2O	—	123.0	—	4.0
70	NaCl	—	161.4	—	—
70	$CaCl_2$	42.0	61.4	44.0	52.0

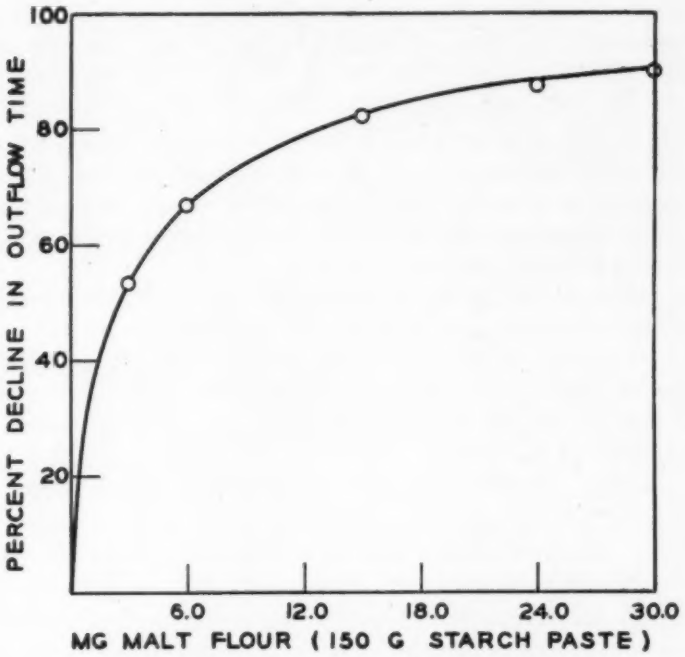

Fig. 6. Decline in outflow time of the starch paste when liquefied by unheated malt extract.

tion used for liquefaction only alpha-amylase was effective, whereas *both* alpha- and beta-amylase are effective in dextrinization. This would explain the results obtained by Lüers and Rümmler (1935) in which they showed that heating affected dextrinizing activity but had little effect upon liquefying activity. This would most likely explain also why these authors were unable to obtain correlation between these two activities in their partial inactivation of barley malt with acid and ultraviolet light. Very little correlation would be expected between the two activities as long as the partial heat-inactivation affected the beta-amylase and this amylase affected the iodine values but not the liquefying values. The elimination of this factor by the use of alpha dextrinizing values of the malt brought about better correlation, as is shown in Table VII.

It is noted that the amount of inactivation upon heating, as measured by the liquefying activity shown in Table VII, is greater than that shown in Table VI for the same conditions. This is no doubt due to the fact that more dilute solutions of the malt were heated in the cases using excess beta-amylase, and the amount of inactivation of the alpha-amylase of a malt solution upon heating is dependent upon the concentration of the malt extract heated.[5]

[5] The effect of concentration of protein and the presence of various ions upon the heat inactivation of malt is being investigated in this laboratory.

Since the correlation between the dextrinizing and the liquefying activities after partial thermal inactivation is high for all three enzymes, the conclusion may be drawn that the two activities are functions of the same enzyme. The calcium ion is shown to be a protective factor against heat inactivation in all three cases and it shows the same amount of protective action towards both activities. This is further evidence that liquefaction and dextrinization are affected by the same amylase. The amylases heated in the presence of the sodium ion seem to be inactivated more than when heated in water alone.

Parallelism in the effects of changes in acidity on liquefaction and dextrinization: It has long been known that amylase activity is profoundly influenced by degree of acidity. Any variation from a certain optimum (dependent upon the amylase) produces a decided decrease in enzyme activity. All of the previous determinations were made at a pH of 4.7, which was chosen as an arbitrary and convenient working acidity. In this particular phase of the work the pH was varied and the effects of this variation on the liquefying and dextrinizing activities were determined. A tendency towards parallelism would be expected in agreement with the previous observations.

The pH was varied by means of buffer solutions with different ratios of acetic acid to sodium acetate. The total concentration of the acetate ion was kept constant. The same ratios of bacterial amylase, taka-diastase, and malt alpha-amylase to starch were used as in the preceding studies of dextrinizing and liquefying activity.

The pH of each starch sample was determined before and after the enzyme treatment by use of the Coleman pH electrometer. It was found that the values sometimes varied slightly between the two determinations. This is most likely due to the difference between the buffering action of the starch and that of its degradation products. However, in the data found in Table VIII only the pH values before the enzyme action are given.

The results found for the effects of the change in the acidity upon the liquefying and dextrinizing activities of the three amylases are tabulated in Table VIII.

In general there is a strong tendency towards parallelism with this type of comparison. The correlation is much higher between the two activities in instances where the pH was not greatly different from the optimum.

The bacterial amylase showed a progressive decrease in activity as the hydrogen-ion concentration of the substrate was increased. Taka-diastase and malt alpha-amylase showed comparatively less variation in activity in the pH range selected, since the optimum in both cases fell towards the intermediate pH values. Since the decrease in activity with

TABLE VIII

DETERMINATION OF THE RELATIVE LIQUEFYING AND DEXTRINIZING ACTIVITIES OF THE THREE AMYLASE PREPARATIONS AT VARIOUS pHs

pH	Bacterial amylase		Takadiastase		Malt alpha-amylase	
	Dextrinizing activity	Liquefying activity	Dextrinizing activity	Liquefying activity	Dextrinizing activity	Liquefying activity
	%	%	%	%	%	%
6.1–6.2	100	100	81	75	75	81
5.2–5.3	95	95	100	100	100	100
4.9–5.0	88	94	100	100	100	100
4.4–4.5	70	84	96	92	95	97
4.2–4.3	50	70	93	89	93	90
3.4–3.5	—	10	72	70	20	28

variation of pH was not as great in the latter two instances, it is to be expected that the inactivation of the enzymes would not be as much of a factor, and higher correlation between the activities would be obtained.

Summary and Conclusions

The dextrinizing and liquefying functions of amylases from three different sources (malted wheat, *Aspergillus oryzae,* and a bacterial preparation) were studied. Malt dextrinizing power was measured by a Wohlgemuth iodine method, alpha dextrinizing activity by the method of Sandstedt, Kneen, and Blish (1939) and liquefying power by the viscometric procedure of Józsa and Gore (1930).

When extracts of the three enzyme preparations were adjusted to an equal basis in terms of alpha dextrinizing power their liquefying activities also were found to be substantially the same. The two types of activity appear to be equally affected by heat and by changes in pH. Calcium ions seem to protect both functions equally against heat inactivation. This consistent parallelism of behavior justifies the conclusion that both the liquefaction and the alpha dextrinization of starch paste by amylases are attributable to the action of *one enzyme,* alpha-amylase.

Since it appears that either the Sandstedt, Kneen, and Blish or the Józsa and Gore viscometric method may reliably be used for the estimation of alpha-amylase activity, it is appropriate to consider the distinctive features of each method, in the interests of establishing a preference for one or the other. A critical and somewhat undesirable feature of the viscometric method is the preparation of starch pastes having standard and constant properties. The mechanical treatment in the preparation of these starch pastes is a factor of vital importance, requiring considerable time and careful manipulation. For a specified duration of time the degree of liquefaction is not a linear function of the quantity of amylase present; hence a reference curve must be established and used for calculating results.

The modified Wohlgemuth iodine method is perhaps more convenient than the other one and more readily adaptable to general standardization. It is primarily a chemical, rather than a physical, method. It requires less equipment and is less time-consuming than the viscometric method. The time required to reach the iodine end-point is a linear function of the amount of amylase present. The Wohlgemuth end-point represents a far more advanced stage of starch degradation than is true of the values obtained in the viscometric procedure.

A notable difference between the methods arises from the fact that beta-amylase is an influencing factor in the Wohlgemuth method unless an excess is added as in the Sandstedt, Kneen, and Blish procedure. However, under the conditions and specifications found most convenient for the operation of the starch-liquefaction method, beta-amylase was not a factor of significance. This is due to the relatively far greater enzyme *dilution* that must necessarily be used in this method. If the concentrations used were equal to those specified for the Wohlgemuth iodine method it is quite probable that beta-amylase would be a factor influencing the rate of liquefaction, as was indicated by trials in which large amounts of beta-amylase were used.

Amylases from different sources may show certain differences in properties. Thus the amylase of bacterial origin shows a higher optimum pH range and a greater resistance to heat than do the amylases of malted wheat and of *Aspergillus oryzae*.

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FURTHER STUDIES ON THE RETARDATION OF THE STALING OF BREAD BY FREEZING

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Recent experiments on the freezing of bread by Cathcart and Luber (1939) showed that the development of an "off" aroma was the principal factor limiting the time that bread could be kept salable by freezing. Cathcart and Luber's work showed that bread in sharp freezers at —22°C remains salable for about 40 days. Bread stored at —22°C for longer periods was considered unsalable because of the development of this "off" aroma. Bread stored in freezers at temperatures higher than —22°C developed this aroma sooner and that stored at lower temperatures developed it more slowly.

Cathcart and Luber pointed out two reasons which might account for the development of this aroma: activity of enzymes of microorganisms at low temperatures and oxidation of proteins and fats. The first cause is probably of little significance because of the high baking temperatures employed. The temperatures are such that they would inactivate the enzymes and destroy most microorganisms in the loaf, only a few spore formers being able to survive. Cathcart and Luber were also able to rule out any effect of absorbed odors from the papers used for wrapping, since the off aroma developed in unwrapped bread.

Also, the intensity of the aroma did not seem to be greater in the unwrapped samples, thus more or less eliminating the possibility of its being absorbed from the commercial freezer.

The following experiments were conducted in an attempt to determine the cause of the development of the off aroma, as well as to seek ways and means of eliminating it.

Quicker Freezing

In order to find out if quicker freezing methods would delay the development of this off aroma, one such method was tried. Commercial bread was frozen in a freezing tunnel at —22°C and at —35°C. In both cases wrapped and unwrapped breads were frozen. Moisture-proof waxed paper wrappings were used for wrapping all frozen bread. The freezing curves obtained by inserting thermometers into the center of the loaves, and reading them at intervals, are given in Figure 1. The

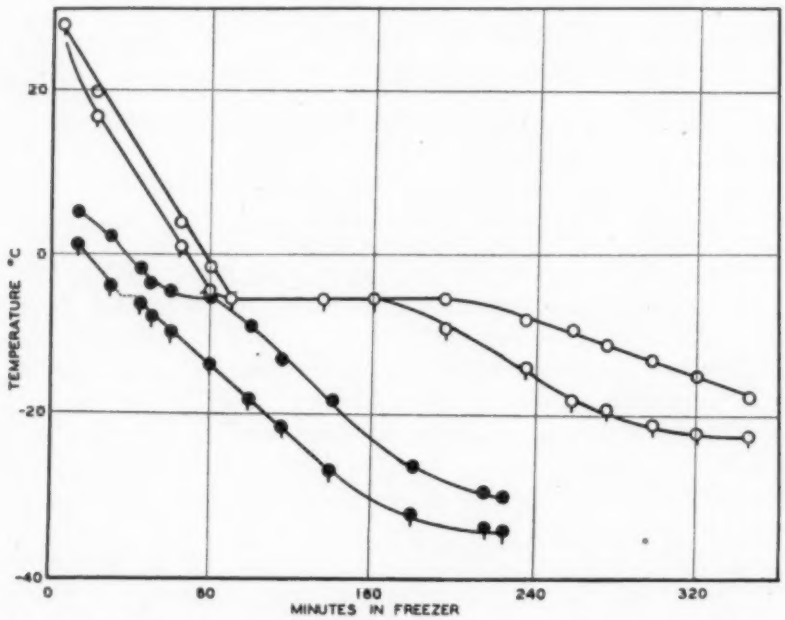

Fig. 1. Rate of cooling of bread in forced-air tunnel. Open circles represent wax wrapped, —22°C; open circles with suspended bar, unwrapped, —22°C. Closed circles represent wax wrapped, —35°C; closed circles with suspended bar, unwrapped, —35°C.

flat portion of the curve at about —5°C represents the latent heat of freezing of the bread. The comparison of these curves with those reported by Cathcart and Luber (1939) shows that the wrapped bread in the tunnel froze faster than that in still air at the same temperature.

However, the unwrapped bread froze at still a faster rate, especially at a temperature of —35°C.

The bread frozen at —22°C was stored at —22°C, while the bread frozen at —35°C was divided into two portions, half of it being stored at —35°C and half stored at —22°C. Samples of the same bread were put into individual freezers (—22°C and —35°C) and frozen in still air. As soon as the unwrapped loaves were removed from the freezing tunnel they were wrapped (in the freezer) and the wrappers sealed. All the samples of bread were tested at intervals by the "swelling power" test and by two persons who were experienced in judging bread.

After 30 days in the freezer there was no significant difference either by the "swelling power" test or by organoleptic tests between the bread frozen in still air at —22°C and stored at —22°C, the bread frozen in the freezing tunnel at —22°C and stored at —22°C, and the bread frozen in the freezing tunnel at —35°C and stored at —22°C. Whether the loaves were frozen in a wrapped or an unwrapped condition made little difference. Tests at longer periods of time indicated that the off aroma developed at equal rates in all of these samples.

The samples frozen in the freezing tunnel at —35°C and stored at —35°C, whether frozen in a wrapped or an unwrapped condition, showed no advantage over wrapped bread frozen and stored in still air at —35°C. As was shown by Cathcart and Luber, however, all samples stored at —35°C kept longer than those at —22°C.

The unwrapped samples frozen in the freezing tunnel showed some drying out. This, of course, is to be expected as a result not only of the unwrapped condition but of the forced draft in the freezing tunnel. All of the "swelling power" tests showed a decrease to a value characteristic of stale bread; then an increase, after 15 or 20 days, to a value of 38 to 40 ml in sediment; and then a decrease again to a value that would be expected for stale bread. This is mentioned since it confirms an observation reported by Cathcart and Luber (1939).

All of the tests and details are not presented since the quicker freezing did not affect the results significantly.

Effects of Ingredients and Antioxidants

In order to test the effect of ingredients, bread was made in accordance with commercial practice, and all ingredients were kept constant except the one to be tested. One set of control loaves was made in the normal manner with all the common enriching ingredients. Another set contained only flour, salt, yeast, and water. Another contained all the ingredients except dry skim milk, another all the ingredients except

shortening, and another all ingredients except malt extract. Other loaves were made with 1%, 2½%, and 5% of Avenex No. 7 (specially milled oat flour). Some of the control loaves were wrapped with a special glassine avenized (oat-flour-treated) paper before the moisture-proof wax was applied. All of the loaves were wrapped in wax paper in the commercial manner and put in a sharp freezer at —22°C.

Samples of the various breads were taken from the freezer at intervals, peroxide values determined, and organoleptic tests made. The peroxide values were determined on residues obtained by extracting a ground sample of the bread with ether and evaporating off the ether on a water bath. The technique used was essentially that of Wheeler (1932). Every effort was made to handle the peroxide procedure in exactly the same way at all times so that the results would be strictly comparative. The organoleptic tests were again made by two persons experienced in judging bread. The loaves were tested at intervals for a period of three months after they had been placed in the freezer.

There was no significant difference, either in peroxide values or organoleptic flavor tests, between the loaves made with the normal formula (containing enriching ingredients), the lean formula, the one without milk, the one without shortening, and the one without malt. According to organoleptic tests the off aroma developed at about the same time in all of them.

Although the peroxide values on the loaves containing oat flour and those wrapped in the oat-flour-treated paper were slightly lower in general (with considerable variations) than the values on the normal loaves, the organoleptic tests did not show significant differences. According to the scorers, the development of the off aroma came at about the same time, whether the loaves contained oat flour, were wrapped in oat-flour-treated paper, or were simply standard loaves wrapped in moisture-proof wax wrappers. Thus, since neither variations in formula nor the use of oat flour as an antioxidant made any significant difference in the results, the detailed data are not presented.

Canning of Bread

Since the above results gave some indication that oxidation might at least be one factor in the development of the "off" aroma, it was decided to freeze and store some bread that had been sealed hermetically. Three samples of commercial bread were used: one sample was sealed in wax wrappers, one was sealed air-tight in a standard commercial tinned can, and the third was vacuum packed in a tinned can. The bread was packaged three hours after being baked. It was then immediately placed in a sharp freezer at —22°C and stored. Samples were

tested at various periods of time by the "swelling power" test and by organoleptic flavor tests.

These tests are summarized in Table I. Since the results on the control loaves wrapped in wax paper were the same as those reported by Cathcart and Luber (1939), the tests on these loaves are not included in the table. It will be noted from the table that both samples remained in good condition and were salable up to the 345-day test. At 565 days both samples showed a decrease in quality, the crumb color becoming darker and more damp to the touch. However, even at 565

TABLE I

TESTS ON WHITE BREAD CANNED AND FROZEN AT − 22° C FOR VARIOUS PERIODS OF TIME

(Bread was three hours out of oven when canned and placed in freezer. Four hours allowed for thawing before opening and testing.)

Series No.	Time in freezer	Canning process	Swelling power	Score		Remarks
				Aroma 15	Taste 20	
	days					
1	21	Hermetic Vacuum	34 34	14.0 14.0	19.0 19.0	All characteristics same as fresh bread.
2	60	Hermetic Vacuum	32 32	13.5 14.0	19.0 19.0	Crust character and aroma slightly better on vacuum sample. Vacuum sample same as fresh bread.
3	160	Hermetic Vacuum	39 35	13.5 14.0	19.0 19.0	Crust character and aroma slightly better on vacuum sample. Vacuum sample same as fresh bread.
4	345	Hermetic Vacuum	34 36	13.5 14.0	19.0 19.0	Vacuum sample slightly better. Aroma slightly stronger on both samples.
5	565	Hermetic Vacuum	34 34	12.5 13.0	18.0 18.5	Both samples showed a decrease in quality. The crumb color of both was dull.

days there was little evidence in either sample of the off aroma that had developed in the uncanned bread. The comments and remarks of the judges show that the sample packed in vacuum was slightly preferable to that simply sealed in a can at normal atmospheric pressure. The "swelling power" values showed little variation throughout the test; however, there is an indication of an increase in the values at the 160-day test.

These experiments on the canning of bread indicate that the development of the off aroma is at least to some extent due to oxidation, and

that by preventing this oxidation (in this instance by canning), the salability of bread frozen and held at —22°C can be increased from about 40 days to at least 345 days. The process of vacuum canning the bread before freezing not only increases the length of time that the bread remains salable but keeps the bread in excellent condition for a period of about one year.

Since quick-frozen foods in cans are showing possibilities of success, it may be that the canning and freezing of bread for storage over long periods of time and for special purposes will prove practical in the future. In commercial practice the canning of bread is not new. There have been references to canned bread in the bakery trade journals for the past fifteen years. The most recent and comprehensive report on the canning of bread was given by Pfannenstiel and Salomon (1939). They did not stress the keeping qualities of the bread but noted that when the bread was baked in air-tight cans it was crustless, and the crumb had the same properties, including moisture content, as ordinary bread. They pointed out that the acidity was higher, the vitamin content higher, that complete sterilization was attained, and that consumer tests gave highly satisfactory results.

Published reports generally are concerned with the baking of bread in cans and preservation at room temperatures after baking. Most workers claim a long keeping time for bread baked by this process. Experiments conducted here at the Institute indicate that such bread would have a long keeping time as far as its moisture content is concerned, but staling, due to starch conversion, goes on very rapidly. Thus the bread loses its freshness as judged organoleptically and becomes more solid, just as it would if it were in a wax wrapper (this was verified by experiments). However, if bread were baked in the can and then frozen, it would undoubtedly keep in excellent condition for at least a period of one year, and perhaps longer, on the basis of results reported above. Of course the advantage of the procedure as carried out in this work is that it produces bread that is identical with commercial bread as offered to the trade.

Summary

The following methods have been used in attempting to increase the length of time that bread can be kept fresh and salable by freezing at —22°C or —35°C by sharp freezing methods as employed by Cathcart and Luber (1939): (1) quicker freezing by employing a freezing tunnel, (2) variations in formula and use of oat flour as an antioxidant, (3) hermetically sealing in the normal atmosphere and in vacuum, employing modern canning technique, before freezing.

The quickest method of freezing employed reduced the bread to the solid state (internal temperature —10°C) in 60 minutes. This represented a reduction in freezing time of at least 60 minutes over the quickest method employed by Cathcart and Luber in their sharp freezing experiments. However, the quicker freezing method did not show a significantly longer keeping time for the bread than sharp freezing at the same temperature.

Variations in the formula or the use of oat flour as an antioxidant (directly in the product or on the wrapping paper) did not lengthen the keeping time significantly according to organoleptic tests, although oat flour showed a slight lengthening of the keeping time according to peroxide values.

The baking and subsequent canning (hermetically sealed in normal atmosphere and in vacuum) gave the bread a greatly increased keeping time. The bread sealed in the normal atmosphere remained salable for a period of approximately one year, while that in the vacuum not only remained salable but remained quite similar to fresh bread for this same period.

From the practical standpoint of commercial bread production in the United States at the present time, this lengthening of the keeping time of frozen bread may not be of great importance. In fact, it is felt that the keeping time reported by Cathcart and Luber for frozen bread in moisture-proof wrappers is sufficient. Actually, however, it throws light on the cause of the off aroma that develops in frozen bread and should help to clarify the cause of so-called " stale " aroma that develops in time in ordinary bread that is not frozen. The canning and freezing of bread may also prove of value in cases of emergency.

Acknowledgment

The author wishes to express his appreciation to Armour and Company and to Swift and Company for the use of their freezers and equipment, and for the advice given by their staff members in regard to this problem.

Appreciation is also given to the Quaker Oats Company for furnishing the oat flour (Avenex) and to the Musher Foundation, Inc., for obtaining the avenized paper, which was used in these experiments.

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A COMPARISON OF HARD RED SPRING AND HARD RED WINTER WHEATS [1]

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(Read at the Annual Meeting, May 1940)

The relative baking qualities of hard red winter and hard red spring wheats has long been a subject of much debate, and little agreement. It has been rather generally accepted abroad that the hard red spring wheats are superior to the hard red winter wheats and the persistent spread in prices of the two classes of wheat and of flours milled from them has tended to establish this belief. In a study of published data relevant to this subject, Larmour (1940) concluded that the data presented did not warrant this conclusion. There was abundant evidence that the two classes differ in certain characteristics, but in regard to intrinsic baking quality there was no consistent support of the idea that one class is superior to the other.

It is possible, and indeed likely, that the experimental methods applied in studies of these two classes of wheats were inadequate to evaluate certain factors that a commercial baker might be able to recognize but which he could not describe quantitatively. Fisher and Jones (1937) in discussing the characteristics of the wheats of commerce, speak a good deal of the "tenderness" of doughs, a quality which is recognizable by the practical baker, but one which has thus far defied all attempts at accurate measurement. In describing flours milled from American hard winter wheats, they state that "such flour has a high water absorption but not so extensive a fermentation tolerance as that from Marquis, and though giving an excellent loaf when baked alone, has less carrying power for weak flours." They regard the hard winter wheat, as received in Great Britain, as being in the same category as the Plate wheats from Argentina; that is, they neither add to nor detract from the quality of a blend, and are therefore to be regarded as "filler" wheats.

A great many people in the United States, especially those in the southwest who have the opportunity to get the wheats and flours at first hand from the areas of production, do not agree that this is a correct and general description of American hard winter wheats. They think that the hard winter wheats procured from areas that are suited climatically to produce high-protein wheat make flour equal in baking qual-

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ity to the spring-wheat flours. Indeed there are some who would go as far as to maintain that the hard winter wheat flours are actually superior to all others for commercial bakeshop practice. Larmour, Working, and Ofelt (1939) reported the results of baking tests made with the principal varieties grown at that time in the southwest. While no direct comparison with spring-wheat samples of comparable protein content was made, it was clearly evident from their data that the relation between loaf volume and protein was linear, and that the volumes were of the same order as would be expected from hard red spring wheat flours of comparable protein. It was thought at the time that this might have been attributable to the baking formula used, a rich formula with shortening and milk, which might have exerted a bolstering effect on the flours. It was decided to investigate this point at the earliest opportunity with both hard winter and hard spring flours. In the fall of 1939 some hard winter samples were procured from Kansas through the courtesy of Dr. J. H. Parker, and these were compared with a number of typical hard spring varieties grown in Saskatchewan, and selected on the basis of previous baking results. Both lots were of the 1939 crop.

Experimental

All the samples were milled to a straight-grade flour of approximately 70% extraction, on a three-stand Allis-Chalmers experimental mill. After aging for five weeks, the flours were baked by six baking formulas. Formula I was the "malt-phosphate-bromate" formula, consisting of the A.A.C.C. basic formula plus malt extract 0.3%, ammonium dihydrogen phosphate, 0.1%, and potassium bromate 0.001%, with standard fermentation, proof, and pan times, and temperatures. Formula II was a rich formula with yeast 3%, sugar 5%, salt 1.75%, shortening 4%, dry milk solids 4%, and malt, phosphate and bromate as in Formula I. The fermentation time was the same as above, but the pan proofing was done at 35°C for 55 minutes. Baking times and temperatures were the same for both formulas and were those set forth in the standard method. Four modifications of Formula II were used: no bromate, 2 mg, 3 mg, and 4 mg.

All doughs were mixed in a Hobart mixer equipped with two hooks; each 100-g batch was mixed separately. They were mixed at slightly variable times, until a smooth dough was obtained. All punching was done by means of National "pup" sheeting rolls. In panning, the doughs were first passed through the sheeting rolls twice and then rolled up by hand. Low-form tins were used for baking.

The baking results obtained by the malt-phosphate-bromate formula are given in Table I. The relation of loaf volume to protein content of the flour is shown graphically in Figure 1.

TABLE I

BAKING DATA FOR HARD RED SPRING AND HARD RED WINTER WHEAT VARIETIES 1939—MALT-BROMATE-PHOSPHATE FORMULA

	Variety	Flour protein	Absorption	Loaf volume	Texture	Crumb color	Baker's comments
		%	%	*ml*	*score*	*score*	
1	Thatcher	16.7	66	1210	7 open	8.5	
2	Thatcher	14.9	67	1050	6 open	8.5	
3	Thatcher	13.6	66	880	8	8.0	
4	Thatcher	11.8	66	750	8	8.0	
5	Thatcher	10.8	66	695	8	8.0	
6	Renown	15.8	66	985	8.5	7.5	Renown samples all quite open.
7	Renown	14.4	64	915	7.5	7.5	
8	Renown	13.2	64	815	6	7.5	
9	Turkey	12.7	62	750	7	5.0	
10	Kanred	15.2	63	850	7.5	6.0	
11	Tenmarq	15.0	65	1010	7	7.5	Strong flour.
12	Blackhull	13.4	62	820	7.5	7.5	
13	Superhard Blackhull	15.1	65	740	7.5	7.5	
14	Chiefkan	11.3	63	580	4	6.0	Runny.
15	Cheyenne	14.5	64	900	9	9.5	Strong flour.
16	Nebred	15.4	64	1165	7	8.5	Strong flour.
17	Kawvale	9.6	60	570	5	6.0	
18	Clarkan	11.3	56	635	4	7.5	Short, dead.

It should not be regarded as surprising that the hard red spring wheat flours exhibit such linearity of loaf volume in relation to protein content, because these samples were selected from a large number previously baked, and they were chosen from the regression line of loaf volume on protein. They may be regarded as being very typical of Saskatchewan samples of the crop of 1939. The hard red winter samples, on the other hand, were single samples chosen virtually at random, and therefore would not be expected to show as direct a relationship between loaf volume and protein as the others even if they were all of one variety. Actually there were eight different varieties of hard red winter wheats and two soft red winter wheats in this series.

The two varieties of spring wheat were chosen because these two varieties, Thatcher and Renown, represent roughly the range of differentiation allowable for varieties in western Canada. It is well recognized that Thatcher is a very high quality variety, stronger than Marquis on the whole, but in view of the fact that it does not represent more than 50% of the total hard spring wheat production in Saskatchewan, it is scarcely to be considered fully representative of the spring wheat of western Canada at present.

Figure 1 shows that four of the ten winter-wheat varieties were distinctly inferior to the hard red spring wheat flours in loaf volume by the malt-phosphate-bromate formula. These four included Kanred, Super-

hard Blackhull, Chiefkan, and the soft wheat Clarkan. The others, Turkey, Tenmarq, Cheyenne, Blackhull, Nebred, and Kawvale, appeared to fall into places that would be expected from consideration of the hard

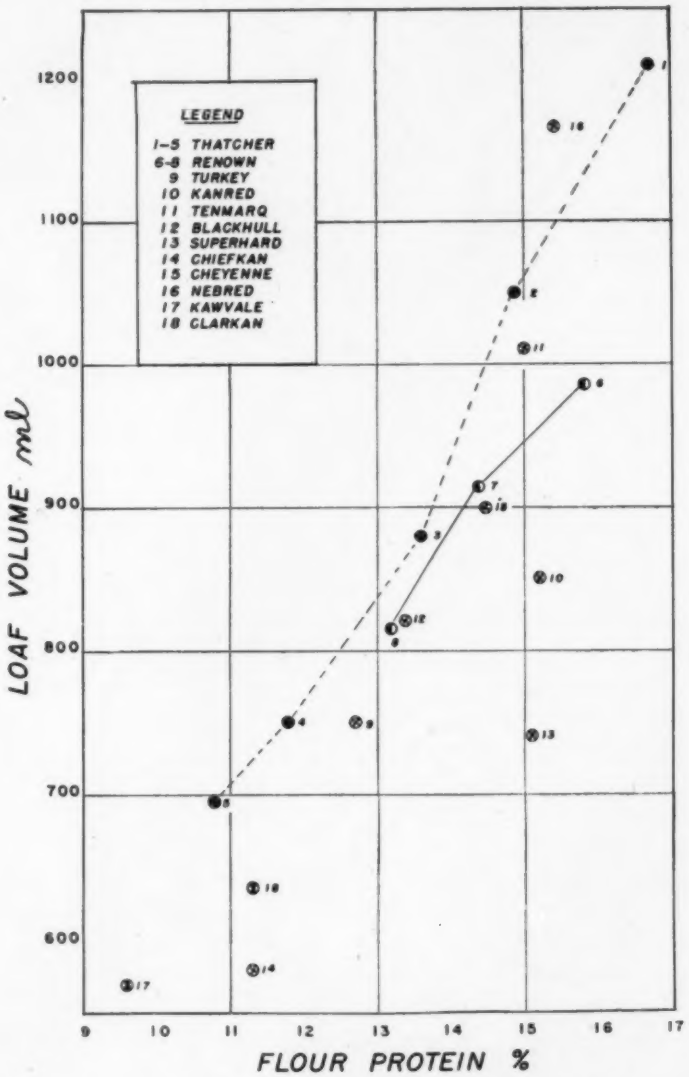

Fig. 1. Relation of loaf volume to flour protein, Formula I.

spring wheat samples. This was rather surprising because Formula I was developed particularly to show strength in the spring wheats, and from *a priori* consideration would hardly be expected to be well adapted

to evaluating another class of wheat which differed essentially in characteristics. However, it may be recalled that Bayfield used Formula I with considerable satisfaction in the evaluation of the soft wheats of Ohio.

With regard to other characteristics, it may be mentioned that the baker commented that the doughs from Tenmarq, Cheyenne, and Nebred felt and behaved during fermentation like "strong" flour doughs, by which is meant that they handled like the spring wheat flour doughs to which he had been accustomed. Careful examination of the crumb texture and grain failed to reveal any outstanding or describable differences between the two classes of wheat represented, except in the cases of the two soft wheats and Chiefkan, all of which were of coarser and harsher texture than the others.

It has to be concluded from this baking test that five of the eight hard winter wheat varieties were equal in intrinsic baking quality to hard red spring wheats of corresponding protein content, and that three were conspicuously inferior.

These flours were next baked by the rich Formula II, with various amounts of bromate. Ofelt and Larmour (1940) showed that the hard winter wheat flours naturally require more bromate than the hard spring flours, and it is well recognized that they need stronger dosages of Agene in commercial practice. It was thought desirable therefore to use a range of bromate dosages in order to get the optimum conditions for each flour in the series. The baking data for these bakings are given in Table II. The loaf volumes obtained at optimum bromate dosage are plotted against the protein content of the flour in Figure 2.

It can be seen in Table II that the optimum bromate dosage for Thatcher was 1 mg, for Renown and for most of the hard winter wheats it was 2 mg, although on the whole the differences between loaf volumes obtained with 1 and 2 mg of bromate were small and probably were not very significant.

In Figure 2 it is shown that the positions of Chiefkan and Superhard Blackhull were about as they were with Formula I; in respect to the rest of the samples they are both far below the others. The Kanred showed to better advantage, but the Cheyenne appeared poorer with Formula II than with Formula I. It is interesting to note that the Clarkan sample showed up much better in quality with Formula II. With this method of test baking, all the winter-wheat samples except Cheyenne, Chiefkan, and Superhard appeared to be equal to the Renown samples in quality. The failure of Cheyenne to fall within the range of the other wheats may be due to inadequacy of the mixing. It was reported by Blish and Sandstedt (1935) that wheat of this variety re-

TABLE II

COMPARISON OF HARD RED SPRING AND HARD RED WINTER WHEAT VARIETIES, 1939 —BAKING DATA FOR FORMULA II WITH VARYING AMOUNTS OF $KBrO_3$

	Variety	Loaf volume (ml) at bromate levels of:					Crumb texture at bromate levels of:				
		0	1 mg	2 mg	3 mg	4 mg	0	1 mg	2 mg	3 mg	4 mg
1	Thatcher	1015	1200	1190	1060		9.5	8.5	8.5	8.5	7.2
2	Thatcher	950	1075	1055	975	880	9.5	10.0	8.7	8.5	8.2
3	Thatcher	890	980	955	865	825	8.7	9.5	9.5	8.7	7.5
4	Thatcher	830	850	820	770	735	9	9.7	10.0	9.2	9.2
5	Thatcher	770	815	765	725	700	8.2	9.7	9.7	8.7	8.5
6	Renown	860	1040	1065	975	945	7.2	9.0	8.5	8.5	8.2
7	Renown	835	960	985	915	845	7	8.7	8.7	8.5	8.0
8	Renown	795	900	905	880	785	7	8.7	9	8.5	8.0
9	Turkey	800	860	870	850	850	6	8.5	8.7	9.2	8.5
10	Kanred	750	940	1000	960	960	5	9	9.5	9.2	8.7
11	Tenmarq	885	1000	1015	960	895	8	9.5	10.0	9.5	9
12	Blackhull	895	935	965	950	920	7.5	9.0	9.5	9.3	9
13	Superhard Blackhull	685	835	870	875	880	3.5	8.7	9.0	9	8
14	Chiefkan	615	665	650	680	662	3.5	5.5	6.7	7.5	6.5
15	Cheyenne	885	910	890	835	785	9.0	9.5	9.0	8.7	8.0
16	Nebred	985	1075	1070	1015	1005	8.7	9.5	8.5	8.5	8.5
17	Kawvale	710	750	738	725	720	5	8.2	8	7.5	7.5
18	Clarkan	785	845	865	825	820	7	8.5	8.5	8	8

quired both severe mixing and bromate. The mixing given by the Hobart mixer is hardly severe enough to develop a dough to its maximum. As a matter of fact, it is known from the recording-dough-mixer curves, that the doughs of Cheyenne are taken long before the maximum development has occurred.

Both Chiefkan and Superhard were very much below all the other varieties in quality with all baking formulas. This is in agreement with the results reported by Larmour, Working and Ofelt (1939) and others. There can be little doubt that as far as the ordinary baking technique goes, these two varieties must be regarded as inferior.

Dough-mixing curves of all these varieties were made by means of the National-Swanson micro machine. They are shown in Figure 3. No attempt will be made to associate the curve characteristic with the baking results. A casual inspection will show that very little can be expected from such a study. It can be seen, however, that there was a great deal of differentiation between the winter-wheat varieties, and some distinction between the two spring-wheat varieties. It is interesting to note that Cheyenne and Nebred, both of which gave long mixing maxima somewhat comparable to those of Thatcher, nevertheless were clearly differentiated from the latter by showing a characteristic flattening of the curve in the early stage of mixing. This feature was not found with the hard spring wheats, nor with the other winter wheats.

Tenmarq, which is a cross between Marquis and Turkey, gave a curve having the general characteristics of the Renown curves. The Thatcher samples gave the long smooth type of mixing curve thought to be typical

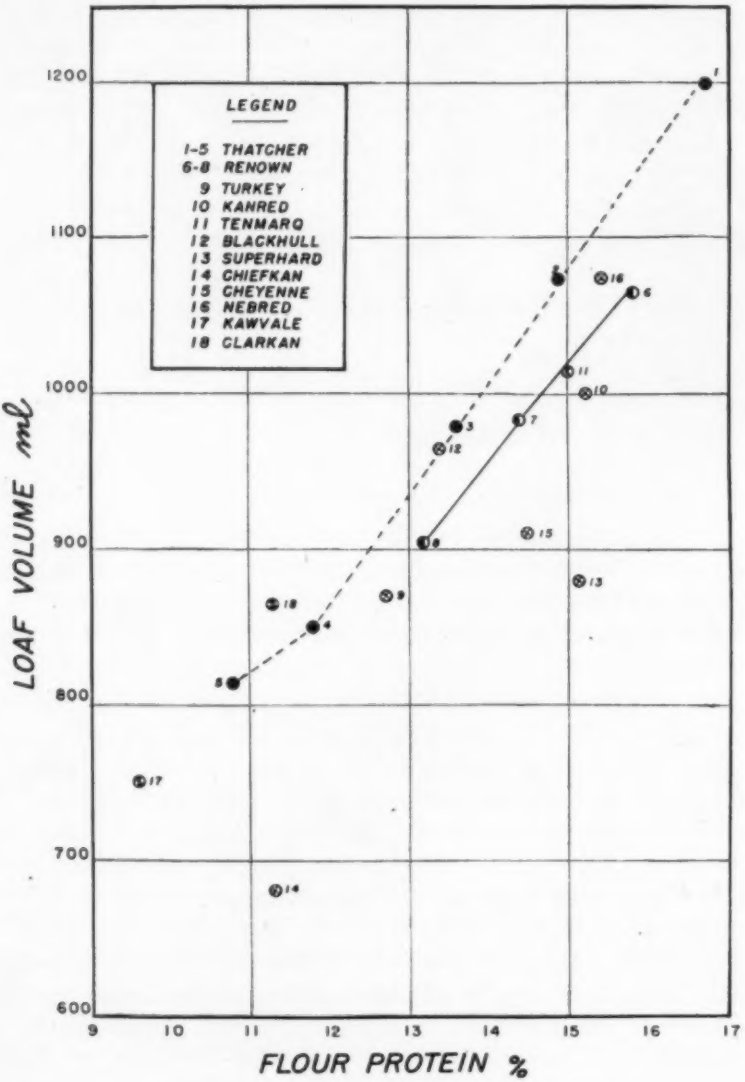

Fig. 2. Relation of loaf volume to flour protein, Formula II.

of the hard spring wheats, although it should be pointed out that as the protein content increases, there is a tendency for the curve to become sharper, a feature that was noted by Larmour, Working, and Ofelt

(1939) in connection with high-protein Turkey, Kanred, Tenmarq, and Cheyenne.

This study has added no new light on the meaning of the mixing curves. They appear to be useful as a means of differentiating varieties on the basis of mixing requirements of the flour-water doughs. There seems to be no doubt that this is a varietal characteristic, and that there is a very useful range of differences between varieties. It is therefore applicable as a means of identifying types of wheats or flours,

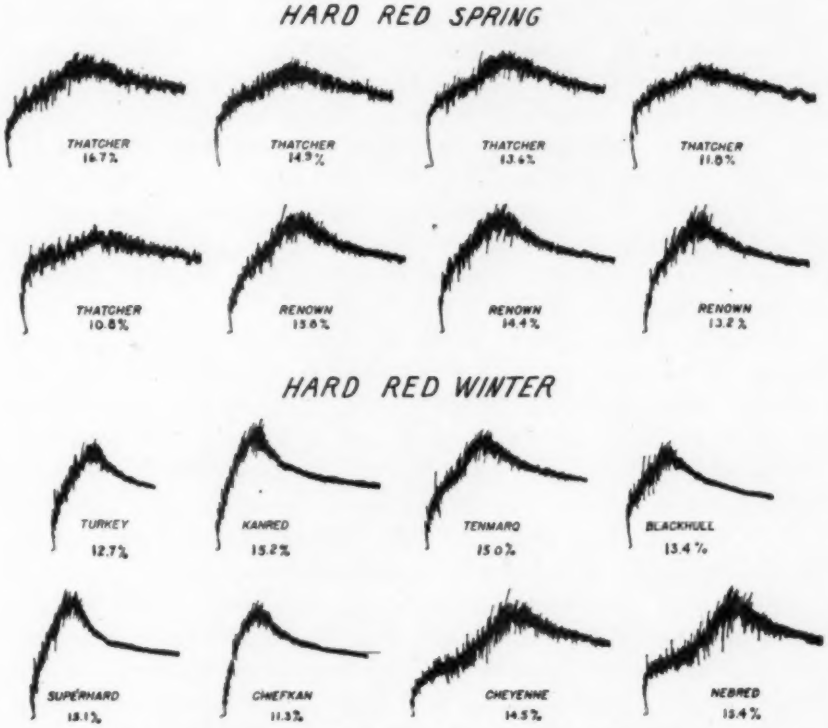

Fig. 3. Mixing curves by the National Swanson-Working recording mixer.

and for certain purposes and in certain areas this identification may be of great importance to the miller and baker, inasmuch as it enables them to choose the type they prefer.

Blending Capacity

While it has been impossible to establish any significant difference between hard red spring and hard red winter flours in loaf volume when they are baked by the ordinary methods, it has always seemed likely that the two classes of wheat might be different in their behaviour when

blended with weaker wheats or flours. It is difficult to know just what the European millers and bakers expect of a blend, and it is still more difficult to obtain the exact sorts of wheat with which the miller customarily blends the American wheats. A thorough study of blending would involve a great many kinds of weak wheat as well as many combinations of the weak and strong components of the blend. It must be realized, therefore, that the experiments reported here represent merely an approach to the problem and the data and conclusions can be regarded as only a preliminary survey.

The flour used as the weak component was a pastry flour milled from soft Ontario wheat. It had a protein content of 7.6% and baked to a volume of about 500 ml with the rich formula. The bread had a heavy, coarse, and wholly undesirable texture. Blends containing 20%, 40%, 60%, and 80% respectively of this flour were baked by Formula II, with 0.001% bromate. Careful observations were made regarding the handling properties of the various doughs in order to see if any distinction could be made on the basis of "tenderness." The baking results are given in Table III, for a number of the samples. Some of the data are shown graphically in Figure 4.

In making comparisons of blends, it is just as necessary to take the protein content into consideration as when one is comparing data obtained on ordinary samples. Consequently, in Figure 4 the loaf volume for each of the blend levels, 20%, 40%, and 60% strong flour, have been plotted against the protein of the blend. The data of each level have been designated by distinctive symbols and joined up by the straight line which seemed to fit the data best. The hard red spring wheat samples are indicated by the more heavily marked symbols.

It should be noted that while the volumes for each blend level have been shown separately, the relation of all the loaf volumes to protein content is as high as might be expected from random samples of one class of wheat. The correlation coefficient of loaf volume and protein content for the 20%, 40%, and 60% blends was + .94. The hard spring samples are quite well distributed within the swarm and show no evidence of being different from the hard winter samples in loaf volume of their blends. The varieties Kanred and Superhard Blackhull fall out of line in the 60% blends.

These data indicate that there may be a tendency for the lower blends to be relatively more effective than the higher blends. This is shown by the somewhat slight but fairly regular displacement of the regression curve to the right as one goes from the lower to the higher blends. If this trend is real, it also indicates that higher-protein wheats are more effective in blends than low-protein wheats, even when the

TABLE III

BAKING DATA FOR BLENDS WITH ONTARIO WINTER WHEAT PASTRY FLOUR

Sample No.	Description of sample	Percent of strong flour	Flour protein	Loaf volume	Crumb texture
		%	%	*ml*	*score*
6	Renown	20	9.3	598	3.5
		40	10.9	751	8
		60	12.5	884	9
		80	14.1	950	10
3	Thatcher	20	8.8	558	3.5
		40	10	650	6
		60	11.2	754	8.5
		80	12.3	875	10
16	Nebred	20	9.2	627	4
		40	10.8	779	8
		60	12.2	885	9.5
		80	13.8	978	10
12	Blackhull	20	8.8	602	3
		40	10.0	655	5
		60	11.0	709	7
		80	12.2	833	9
9	Turkey	20	8.6	557	3
		40	9.7	633	5
		60	10.6	694	7.5
		80	11.7	774	8
10	Kanred	20	9.1	598	3
		40	10.7	702	5.5
		60	12.1	765	7.5
		80	13.7	845	8
11	Tenmarq	20	9.1	605	5.5
		40	10.6	715	8
		60	12.0	820	9.5
		80	13.5	888	10
13	Superhard	20	9.1	605	3.5
		40	10.6	671	5.5
		60	12.1	715	6.5
		80	13.6	750	7
14	Chiefkan	20	8.4	546	1
		40	9.2	594	3
		60	9.8	612	3.5
		80	10.5	614	3.5
15	Cheyenne	20	9.0	602	5.5
		40	10.4	700	7.5
		60	11.7	793	10
		80	13.1	851	10

protein content is taken into consideration. Thus a high-protein flour that yields a blend of 10.9% protein in a 40% blend gives about 35 ml larger loaf volume than a lower-protein sample which in a 60% blend yields a protein content of the same value. This deduction is quite opposite to that of Sandstedt and Ofelt (1940), who in a study of flour-starch blends reported indications of a retrogradation of protein quality with increasing protein content.

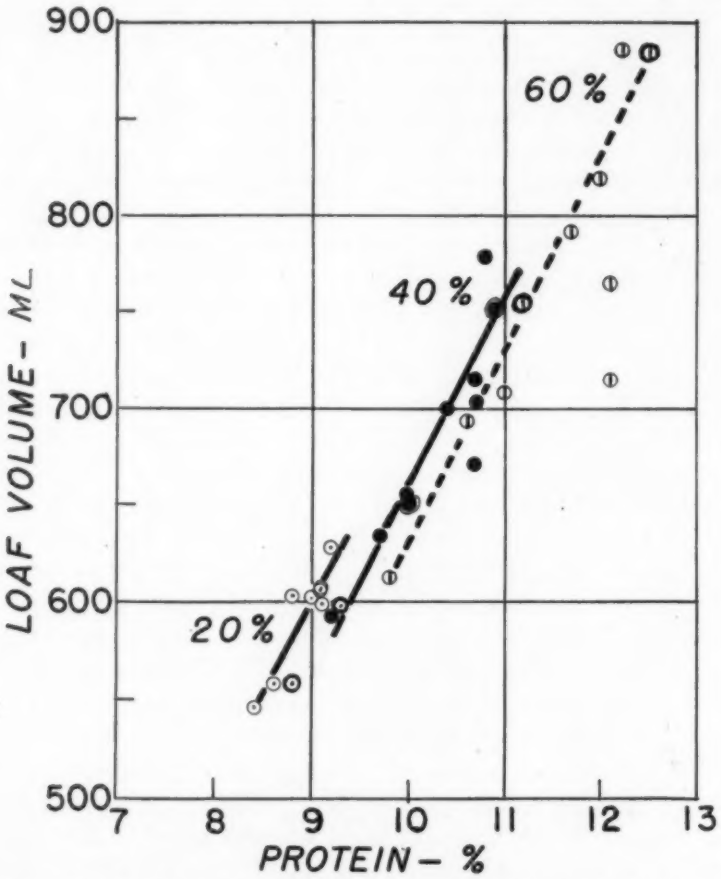

Fig. 4. Relation of loaf volume to flour protein, blends with Ontario soft winter flour.

With regard to handling properties of the doughs, the baker was convinced that there was considerable difference between the hard winter flours and the hard spring flours except in the cases of Nebred and Cheyenne, which he reported behaved like the spring wheat flours. As the descriptive terms used to differentiate the various doughs would have no meaning except for the individual applying them, it was not consid-

ered of any value to include them in this report. The principal criticism of the majority of the hard winter samples was that they produced "tender" doughs. By this was meant that they gave up their gas more readily than the hard spring flours, and showed less tendency to become bucky on handling.

It seems probable that the quality described as "tenderness" might show up better in hearth than in tinned loaves. The softer doughs might be expected to flow out in proofing and give a less bold loaf than the tougher spring wheat flour blends. This possibility was not examined in this study on account of shortage of the supply of the hard red winter flours.

Conclusions

The data presented in this study, although based on a small number of samples chosen at random, seem to support the author's earlier conclusions (1940) based on the published data of numerous workers, that there is no conclusive evidence that the hard red winter wheats are inferior to the hard red spring wheats in intrinsic baking quality, as tested by experimental methods available at the present time.

Tests made with soft wheat flour blends indicated that as far as loaf volume and crumb texture were concerned, there was no distinction between flours of the hard winter and hard spring classes. There may have been a qualitative difference in the handling properties of the doughs, but it was a distinction too elusive to be described with any degree of accuracy.

Dough-mixing curves obtained with the National-Swanson recording mixer show marked differences in the mixing characteristics of varieties of both classes of wheat, but none of the differences recorded seem to be directly related to the baking performance of the flours.

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AN APPARATUS FOR THE MEASUREMENT OF BREAD CRUMB DEFORMATION

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Measurements of the elastic and plastic deformation of bread crumb resulting from the application of weight and pressure are of great interest from both the theoretical and the practical point of view. Indeed a thorough study of dough maturation, bread baking, the evaluation of bread crumb quality, the process of cooling, and storage of kinds of bread is quite impossible without a determination of these properties. It is, however, true that insufficient consideration has hitherto been given to this problem. The reports of Katz (1928), of Platt and Powers (1940), and of some other investigators who studied the bread staling process by observing the changes occurring in the compressibility (total deformation) of bread crumb during the storage period are well known.

In the U.S.S.R. work along these lines was conducted at the All Union Institute for Bread Baking Research (by Kuhlman-Balashova). Bakers have habitually noted the fact that on application of pressure with a finger poorly baked bread crumb will but slightly recover its initial volume, while well baked crumb possesses a better capacity for partial recovery after general deformation. That is why in the standards for baked bread adopted in the U.S.S.R. the following specification for the organoleptic (by feeling) characteristic of crumb is to be found: "Upon application of light pressure on the crumb with a finger, it must quickly recover its original form."

It was while making use of this practice of bread penalization, that Axelrode suggested a special device for determining whether bread is thoroughly baked. This device permitted measurements of the elastic deformation of its crumb.

While studying in 1938–39 the properties of elastic and plastic deformation of bread crumb, the author tested a number of devices employed for this purpose and recognized the need for an improved and more precise measuring apparatus. Since then such an apparatus has been constructed by the Technological Laboratory of the Institute under his supervision. The present paper aims to describe and discuss the new improved apparatus for measurements of elastic and plastic deformation, and the method of its application.

Description of the Apparatus

The structural principle of the apparatus is essentially the same as that of Axelrode but with the following additions and changes:

1. The apparatus is not only suitable for measurements of elastic deformation properties under an unlimited load according to Axelrode's method, but is also applicable to measurements of the total deformation (compressibility) of the crumb.

2. The principle of the action of an unlimited load has been replaced by the action of a definite load within a stated period of time.

3. The sensitivity of the apparatus has been increased approximately 2.5 times that obtainable with the device of Axelrode. This has been done at the expense of increasing the ratio of lever arms—"pressing finger"—and lever measuring pointer.

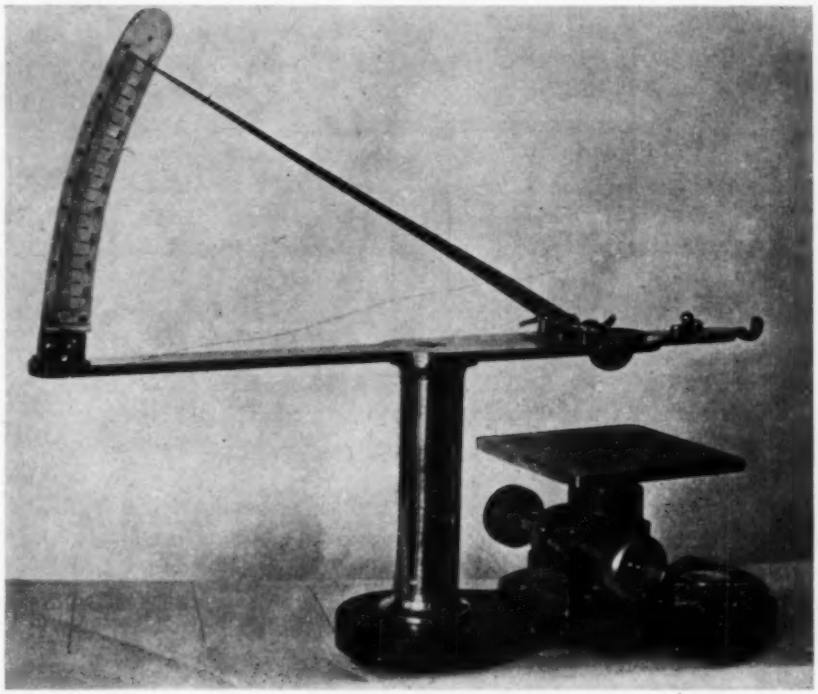

Fig. 1. Apparatus for the measurement of deformation of bread crumb.

4. In contradistinction to the device of Axelrode, which is set directly on bread, the apparatus described is constructed on the principle of the action of only the working member upon an accurately sliced piece of bread crumb of a determined dimension. The apparatus consists of a flat metallic base (*A*) (Fig. 2) with a support (*B*) mounted on it, to which a second flat metallic foundation (*C*) is attached. A

pointer-lever (*D*) and a measuring scale (*E*) are mounted on the foundation (*C*).

The lever is balanced in such a way that there is a light overweight in the shorter arm ending in a semispherical "finger" (*F*) to which the hook (*G*), to hang the weight on, is attached. Consequently the right lever arm is in horizontal position, while the semispherical surface overlaps through the opening by 10 mm beyond the lower base of the apparatus.

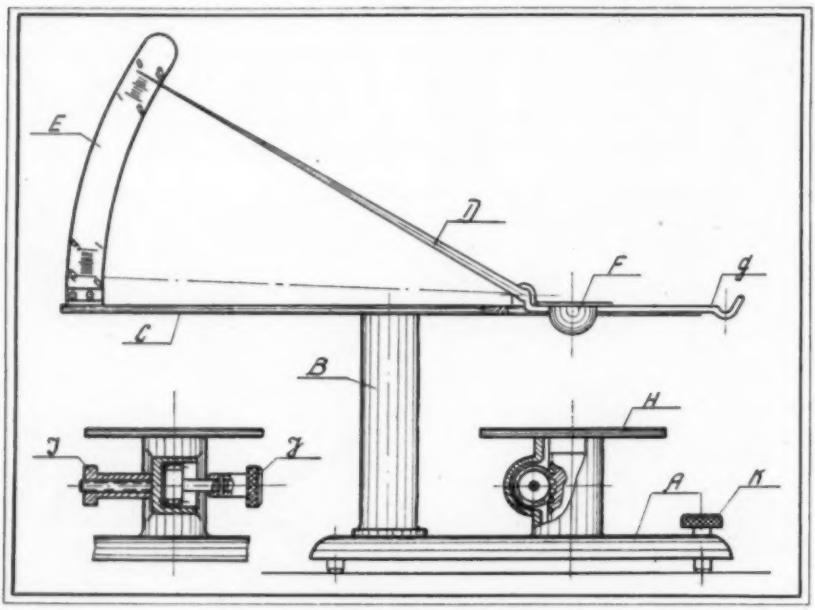

Fig. 2. Detailed drawing of the apparatus.

The distance from the center of the finger semisphere to the point of support is one-tenth of the distance from the support to the end of the measuring pointer.

The left longer lever arm (measuring pointer) usually points to the figure 10 (on the left-hand side of the scale).

Above the lower base of the apparatus (opposite the opening) a lifting platform (*H*) is to be found which with the help of special screws (lifting and stop-screws) may be raised and fixed at the requisite height.

Operation of the Apparatus

The loaf to be tested is divided into two equal parts; then from each half, on the cut side, a slice of 5 × 5 cm and 4 cm thick is cut. In order to reduce errors due to uneven porosity, each loaf is subjected to two

parallel determinations along the central part of the cut. The cut is made with a sharp knife, and the resulting crumbs are carefully removed from the surface of the loaf.

The slice of bread to be tested is placed on the lifting platform, being located in such a way that the central part of the cut is exactly under the opening in the base of the apparatus (in case of irregular porosity in the center, the cut must be slightly shifted). Now with a special screw (*I*) the platform is raised into such a position that the lower surface of the base makes close contact with the upper surface of the cut, the indicating pointer being at zero. Thereupon the platform is fixed in the necessary position by means of the second screw (*J*).

A beaker of waxed paper must be hung on the hook and a measured quantity of water introduced through a pipette into it. The quantity of water depends on the kind of bread being tested and the degree of its staleness. Simultaneously a sand-glass is tilted.

After the expiration of one minute the position of the pointer on the measuring scale is recorded. The position shows the total deformation (compressibility) of the bread crumb. In order to measure the elastic deformation (elasticity) of the crumb, the weight is removed after one minute of load application (the semispherical part of the apparatus touches at that moment the bread surface) thus releasing the pointer, which is left in this position for two minutes more. Under the action of the elastic properties of bread, the "finger" of the apparatus is raised upwards and the indicating pointer goes down.

The difference in the readings taken at the position of the pointer under load and its position two minutes after the removal of the weight characterizes the elastic deformation of the crumb in terms of the scale units. Relative elasticity is calculated as the percentage—ratio of the elastic deformation to total deformation, the latter being taken as 100%.

The difference between duplicate measurements of general compressibility should not exceed 0.5 of the scale units of the apparatus, otherwise a third measurement must be made. Two concordant readings are considered to show compressibility.

Possibilities for Utilization of the Apparatus

Preliminary data on the work conducted by the Technological Laboratory of our Institute with the described apparatus as well as with other devices for measuring bread crumb deformation permit us to outline the possible field of practical utilization of the apparatus and the methods of measurement.

1. Measurements of the bread crumb deformation during heating in the baking process show that well heated (well baked) wheat bread

made of straight flour possesses a definite degree of relative elasticity, which usually is not less than 80%. The rate of compressibility declines as heating progresses, finally reaching a limit. Consequently the degree of maturity of bread or the period of time necessary for baking may be established by an objective method which records the magnitude and the dynamics of relative elasticity, as well as the compressibility of the crumb. These measurements provide possibilities for outlining the more efficient and rational methods of baking and for indicating the economical utilization of ovens.

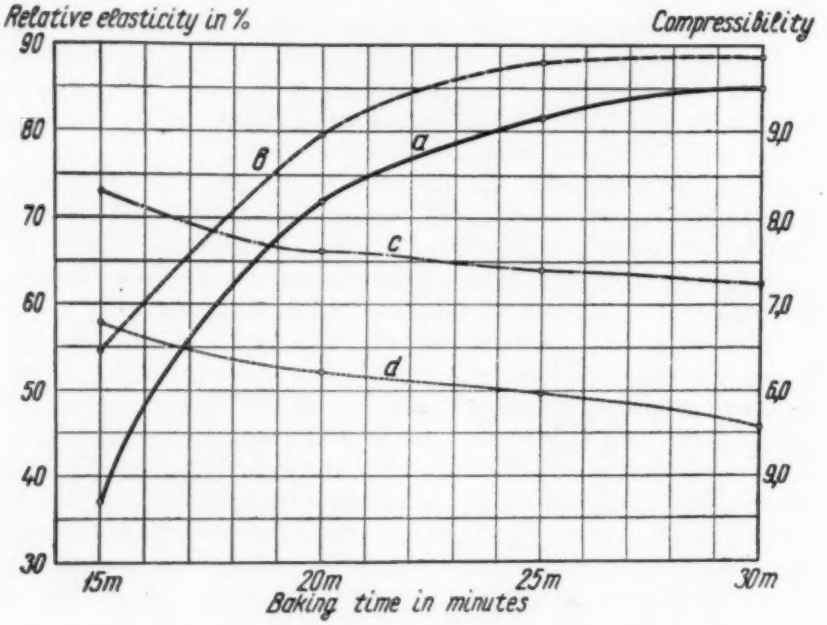

Fig. 3. Changes in the compressibility-elasticity properties of bread crumb—baking time 15–30 minutes: *a*, elasticity 1 hour after baking; *b*, elasticity 6 hours after baking; *c*, compressibility 1 hour after baking; *d*, compressibility 6 hours after baking.

Figure 3 shows values for total and elastic deformation as measured with the apparatus, using straight-flour pan bread. The experiment was conducted in the following way: from the same dough four breads were simultaneously baked with different oven periods (15, 20, 25, and 30 minutes). After one hour of cooling the loaves were cut into two parts; from one-half of each loaf measurements were made of total and elastic deformation and from the second half the same measurements were made after four hours of cooling. The results of the measurements were taken as a basis for two curves, showing decrease of total deformation (compressibility) and increase of elastic deformation as related to length of baking period.

Of great interest is the fact that the elastic properties of insufficiently baked bread showed an improvement upon additional standing after baking. Bread baked in an oven for 15 and 20 minutes showed considerable difference in the magnitude of elastic deformation after one and after four hours of standing. However, even after four hours of standing the total magnitude of elastic deformation was still very low if compared with bread baked during 25–30 minutes. It is also to be noted that the angle of inclination of the curve showing changes in elastic deformation decreased in proportion to the increase of baking period. It shows that after a certain time of baking, the bread reaches a certain practically constant magnitude of elastic deformation, which apparently characterizes the end of the baking period.

2. Insufficiently baked or "doubtful" bread is sometimes left to "season." After a certain lapse of time the bread is again tested for maturity according to the elasticity of the crumb. Preliminary measurements have shown that our apparatus reliably records changes in the elastic properties of bread in the processes of its cooling and storage. Therefore this apparatus enables one to establish the time of seasoning, after which the bread may either be delivered for sale or penalized.

3. Measurements of total deformation provide numerical expression for the quality and the condition of bread crumb and grain. The measurements show that bread crumb with small, well developed thin-wall grain gives a higher magnitude of total deformation (compressibility). If bread is thoroughly baked it will also have high elasticity. Bread with a coarse thick-wall "hornlike" grain shows a low magnitude of total deformation although it may possess high relative elasticity. Consequently when evaluating the "quality" of grain in a bread of a definite grade not only the magnitude of elasticity, but also the compressibility should be duly taken into account.

The magnitude of compressibility and elasticity thus completes an evaluation of the bread baking qualities of selected wheats. Preliminary measurements with this apparatus conducted in the Laboratory for Bread Testing of our Institute (Losev, Zavyalova) have shown that wheats with high baking qualities scored high with respect to total and elastic deformation, while wheats with medium baking qualities showed much lower total deformation and somewhat lower elastic deformation.

4. Investigations conducted by the Technological Laboratory of the Bread Baking Institute seeking to obtain more precise data on minimum seasoning periods for rye-wheat bread prior to cutting it for drying into biscuits proved that this time is also determined by the magnitude of total and elastic deformation. A bread having too large

total deformation (compressibility) and at the same time a low elastic deformation, will be pressed down. For low compressibility (very stale bread) the texture of bread grain seems to be impaired and the biscuits will have small branchlike cracks over the whole of their surface.

5. Finally, the studies of the Technological Laboratory of the Institute have shown that compared with other physicochemical methods the ascertainment of total deformation of the bread crumb in the course of its storage affords the best measurement of the staling process.

The Technological Laboratory of our Institute is continuing the work with a view toward further improvement in precision of the methods for measuring bread crumb deformation.

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1928 Gelatinization and retrogradation of starch in relation to the problem of bread staling. Published as a chapter in R. P. Walton's book "A Comprehensive Survey of Starch Chemistry," The Chemical Catalog Co., New York.
Platt, W., and Powers, R.
1940 Compressibility of bread crumb. Cereal Chem. **17**: 601–621.

THIAMIN CONTENT OF COMMERCIAL WHEATS OF THE 1940 CROP

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It has been pointed out by Schultz, Atkin, and Frey,[2] that there is a considerable range in the level of thiamin in various wheat samples. This study was undertaken to secure further data on the variation that may be expected from wheats of different varieties or from different localities. Because the miller seldom grinds a lot of wheat consisting of a single variety, it was thought that the use of commercial samples would afford a useful basis for making comparisons. Thus most of the wheats analyzed were commercial samples.

Assays were made by the fermentation method of Shultz, Atkin, and Frey.[3] The samples were ground in a Quaker City mill set at the

[1] Present address: National Bakery Division, The Great Atlantic and Pacific Tea Co., New York.
[2] A. S. Schultz, L. Atkin, and C. S. Frey: A preliminary survey of the vitamin B_1 content of American cereals, Cereal Chem. **18**: 106–113, 1941.
[3] Schultz, Atkin, and Frey: A fermentation test for vitamin B_1, J. Am. Chem. Soc. **59**: 2457–2460; The vitamin B_1 content of wheat, flour, and bread, Cereal Chem. **16**: 643–648, 1939.

TABLE I

Sources, Grades, and Thiamin Values of Wheat Samples

Lab. No.	Origin—state, county or locality	Grade and market class	Variety	Test weight	Remarks	Thiamin
				lbs		*μg/g*
W-9	Colo., Denver	2 Dark hard winter	—	—	—	8.0
W-14	Colo., Denver	2 Dark hard winter	—	58.8	—	8.4
W-29	Colo., Denver	2 Dark hard winter	—	—	—	7.7
W-90	Eastern Colo. farm	1 Dark hard winter	—	60.0	—	6.7
W-11	Dakota [1]	3 Dark northern spring	Thatcher	—	—	5.3
W-38	Dakota [1]	2 Dark northern spring	Thatcher	—	—	6.6
W-44	Dakota [1]	2 Dark northern spring	Thatcher	—	—	7.8
W-46	Dakota [1]	1 Dark northern spring	—	—	—	7.7
W-51	Dakota [1]	2 Dark northern spring	—	—	—	7.9
W-57	Dakota [1]	2 Dark northern spring	—	—	—	5.9
W-54	Dakota [1]	3 Hard white	Burbank	—	—	7.5
W-60	Dakota [1]	2 Dark northern spring	—	—	—	5.1
W-24	Dakota [1]	1 Red durum	—	—	—	6.7
W-25	Dakota [1]	2 Hard amber durum	—	—	—	6.4
W-28	Dakota [1]	2 Hard amber durum	—	—	—	6.5
W-31	Dakota [1]	2 Hard amber durum	—	—	—	7.5
W-34	Dakota [1]	4 Hard amber durum	—	—	—	7.7
W-36	Dakota [1]	2 Hard amber durum	—	—	—	6.9
W-40	Dakota [1]	3 Hard amber durum	—	—	—	7.7
W-50	Dakota [1]	2 Red durum	—	—	—	8.0
W-52	Dakota [1]	4 Red durum	—	—	—	8.2
W-41	Dakota [1]	1 Heavy northern spring	—	—	—	5.4
W-66	Idaho, Newdale	1 Dark hard winter	—	62.1	84% dark hard winter	6.4
W-2	Illinois, Pratt County	2 Dark hard winter	—	—	—	5.2
W-16	Illinois, Central	2 Hard winter	—	—	—	5.9
W-45	Illinois, Central	2 Soft red winter	—	—	—	5.9
W-65	Illinois, Central	2 Hard winter	—	—	—	6.9
W-53	Indiana	– Soft red winter	—	—	—	8.0
W-92	Iowa, Council Bluffs	1 Hard winter	—	—	—	7.6
W-85	Kansas, Salina	2 Dark hard winter	Tenmarq	59.6	—	7.2
W-101	Kansas, Gueda Springs	2 hard winter	Tenmarq	59.8	—	6.0
W-105	Kansas, Silver Lake	1 Dark hard winter	Tenmarq and Iowa Red	62.1	14.20% protein	7.4
W-106	Kansas, Clay Co.	1 Hard winter	Tenmarq	60.5	13.35% protein	7.8
W-107	Kansas, Riley Co.	1 Hard winter	Tenmarq	61.1	11.65% protein	6.8
W-108	Kansas, Geary Co.	1 Hard winter	Tenmarq	61.0	12.25% protein	8.2
W-113	Kansas, Riley Co.	1 Hard winter	Tenmarq	61.1	12.50% protein	6.2
W-115	Kansas, Washington Co.	2 Hard winter	Tenmarq	59.2	8% Kawvale	7.7
W-109	Kansas Dickinson County	1 Hard winter	Tenmarq and Blackhull	60.0	12.85% protein	6.4
W-110	Kansas, Russell Co.	1 Dark hard winter	Blackhull	61.1	15.20% protein	7.3
W-116	Kansas, Republic County	1 Dark hard winter	Tenmarq and Blackhull	60.4	13.80% protein	8.0
W-82	Kansas, N. Central	1 Hard winter	Blackhull	63.0	—	6.1
W-114	Kansas, Marion County	2 Hard winter	Chiefkan, Turkey, Tenmarq	58.4	11.85% protein	6.4
W-112	Kansas, Ellsworth County	Sample—Dark hard winter	Turkey and Blackhull	55.6	1% dock, musty odor, 14.55% protein	7.8
W-84	Kansas, N. Central	1 Dark hard winter	Turkey	60.8	—	7.4
W-83	Kansas, N. Central	1 Hard winter	Redhull	62.4	—	5.8
W-4	Kansas	2 Dark hard winter	—	58.0	—	5.1
W-5	Kansas, Wichita	2 Dark hard winter	–	58.1	1.0 dock	6.1
W-10	Kansas	1 Hard winter	—	60.4	—	5.0
W-15	Kansas	2 Dark hard winter	—	58.0	—	6.2
W-21	Kansas Wichita	4 Dark hard winter	—	55.0	—	6.4
W-27	Kansas	1 Hard winter	—	60.0	—	7.8
W-35	Kansas	3 Red winter	—	58.8	8% shrunken and broken	6.4
W-37	Kansas, Wichita	2 Dark hard winter	—	58.1	—	7.8
W-80	Kansas, Salina	2 Dark hard winter	—	59.5	Med. high protein terminal elevator	8.7

TABLE I—*Continued*

Lab. No.	Origin—state, county or locality	Grade and market class	Variety	Test weight	Remarks	Thiamin
				lbs		μg/g
W-81	Kansas, Salina	2 Dark hard winter	—	58.5	Low protein terminal elevator wheat	6.6
W-88	Kansas, Home	1 Hard winter	—	61.4	—	10.2
W-102	Kansas, Arkansas City	2 Hard winter	—	59.0	—	6.3
W-117	Kansas, Seward Co.	2 Dark hard winter	—	59.8	—	7.6
W-118	Kansas, Seward Co.	1 Dark hard winter	—	60.5	—	8.8
W-119	Kansas, Clay Co.	1 Hard winter	—	60.3	—	6.6
W-120	Kansas, Morris County	2 Hard winter	—	59.3	—	8.2
W-121	Kansas, Morris County	2 Hard winter	—	59.6	—	7.9
W-122	Kansas, Dickinson County	2 Hard winter	—	58.0	—	4.6
W-123	Kansas, Shawnee County	1 Hard winter	—	61.1	Protein 12.06, 15% moisture	8.6
W-124	Kansas, Clay Co.	1 Hard winter	—	60.5	Protein 12.81, 15% moisture	6.0
W-126	Kansas, Riley Co.	1 Hard winter	—	61.1	Protein 11.52, 15% moisture	7.4
W-127	Kansas, Riley Co.	1 Hard winter	—	61.1	Protein 12.09, 15% moisture	7.7
W-128	Kansas, McPherson Co.	2 Hard winter	—	59.5	Protein 12.12, 15% moisture	6.9
W-129	Kansas, Shawnee County	1 Dark hard winter	—	62.1	Protein 13.49, 15% moisture	8.0
W-130	Kansas, Geary Co.	1 Hard winter	—	61.0	Protein 11.85, 15% moisture	8.5
W-131	Kansas, Dickinson County	2 Dark hard winter	—	59.5	Protein 13.10, 15% moisture	9.5
W-132	Kansas, Lane Co.	2 Dark hard winter	—	59.3	Protein 15.49, 15% moisture	9.9
W-133	Kansas, Dickinson County	2 Hard winter	—	59.5	Protein 12.46, 15% moisture	8.7
W-94	Kansas, Doniphan County	1 Soft red winter	Harvest Queen	60.5	—	5.8
W-114	Kansas, Shawnee County	2 Mixed—75% hard 25% soft	Tenmarq, Iowa Red (hard) Kawvale (soft)	59.5	—	6.9
W-125	Kansas, Shawnee County	1 Mixed—80% hard 20% soft	—	60.0	Protein 12.37, 15% moisture	5.5
W-62	Maryland	2 Soft red winter	—	—	Garlicky	6.6
W-20	Minnesota	3 Hard winter	Mintuski	—	—	7.2
W-48	Missouri	3 Soft red winter	—	—	—	5.3
W-58	Missouri	– Soft red winter	—	—	—	7.3
W-95	Missouri, Buchanan Co.	1 Red winter	Clarkan	62.0	—	6.3
W-96	Missouri, Buchanan Co.	2 Red winter	Kawvale	58.7	—	5.3
W-97	Missouri, East Central	1 Red winter	Buttercup	60.2	—	6.0
W-98	Missouri, Stewartsville	1 Hard winter	(Turkey Red?)	61.3	"Typical Mo. Yellowberry"	4.1
W-43	Montana	2 Northern spring	—	—	—	6.5
W-63	Montana	1 Dark northern spring	—	—	—	7.0
W-3	Nebraska, Western	3 Dark hard winter	—	—	—	6.6
W-13	Nebraska, Eastern	1 Hard winter	—	—	—	6.9
W-22	Nebraska, Eastern	1 Hard winter	—	—	—	7.3
W-23	Nebraska, Eastern	2 Hard winter	—	—	—	6.5
W-89	Nebraska, Eastern	1 Dark hard winter	—	61.8	—	7.4
W-91	Nebraska, Eastern	3 Hard winter	—	57.2	—	9.4
W-93	Nebraska, Eastern	2 Hard winter	—	59.0	—	9.2
W-134	Nebraska, Plymouth	2 Dark hard winter	—	58.8	1% damaged	7.8
W-39	Ohio	– Soft red winter	—	—	—	5.8
W-42	Ohio	– Soft red winter	—	—	—	6.6
W-47	Ohio	– Soft red winter	—	—	—	6.8
W-49	Ohio	– Soft red winter	—	—	—	6.1
W-59	Ohio, Eastern	3 Soft red winter	—	59.0	4.8% damaged	5.9
W-61	Ohio, Western	2 Soft red winter	—	59.5	2.5% damaged	5.1
W-56	Ohio, East Central	1 Soft red winter	—	60.0	1.9% dockage	6.6
W-6	Oklahoma	1 Dark hard winter	—	60.4	—	6.2
W-7	Oklahoma	2 Hard winter	—	—	—	5.4
W-8	Oklahoma	2 Hard winter	—	60.7	3.2% damaged	5.9
W-19	Oklahoma	2 Hard winter	—	59.0	—	6.0
W-26	Oklahoma	2 Hard winter	—	59.2	—	5.1
W-30	Oklahoma	1 Dark hard winter	—	61.3	—	8.2
W-32	Oklahoma	1 Hard winter	—	60.4	—	5.1
W-99	Oklahoma	2 Hard winter	—	60.0	10% soft wheat	7.8
W-100	Oklahoma	2 Hard winter	—	58.7	—	7.2
W-103	Oklahoma	1 Mixed red winter 88% hard 12% soft	—	60.2	—	5.8
W-104	Oklahoma	2 Mixed—75% hard 25% soft	—	59.5	—	5.1

TABLE I—*Continued*

Lab. No.	Origin—state, county or locality	Grade and market class	Variety	Test weight	Remarks	Thiamin
				lbs.		*μg/g*
W-68	Oregon, Klondike	4 Hard white	—	55.0	—	4.6
W-75	Oregon, Biggs	3 Hard white	—	57.0	—	6.0
W-77	Oregon, Miller	3 Hard white	—	57.7	—	6.0
W-76	Oregon, The Dalles	2 soft white	—	58.6	—	7.0
W-1	Texas	1 Dark hard winter	—	60.8	1.9% dockage	5.8
W-12	Texas	2 Dark hard winter	—	60.6	3.8% dockage	7.0
W-17	Texas	1 Dark hard winter	—	60.5	—	5.2
W-33	Texas	2 Dark hard winter	—	59.7	—	7.4
W-86	Texas, Cook Co.	2 Mixed red winter	Mediterranean red	58.0	Not true to variety	6.9
W-87	Texas, Hale Co.	1 Dark hard winter	Turkey	61.5	—	8.1
W-135	Texas	1 Dark hard winter	—	60.6	1% dockage	6.7
W-136	Texas	1 Dark hard winter	—	60.8	1% dockage elevator wheat	8.0
W-137	Texas	1 Dark hard winter	—	61.6	Elevator wheat	7 6
W-138	Texas	1 Dark hard winter	—	60.5	Elevator wheat	8.1
W-139	Texas	1 Dark hard winter	—	61.5	—	7.5
W-140	Texas	1 Dark hard winter	—	61.8	—	7.8
W-141	Texas	1 Dark hard winter	—	61.7	—	7.5
W-142	Texas	1 Dark hard winter	—	60.7	—	4.2
W-143	Texas	1 Dark hard winter	—	61.7	—	6.6
W-67	Utah, Garland	1 Hard winter	—	61.2	—	4.1
W-64	Washington, Waitsburg	3 Soft white	—	56.0	—	4.2
W-69	Washington, Cowship	1 Soft white	—	60.0	—	4.6
W-78	Washington, Hadley	1 Soft white	—	60.5	—	7.4
W-65	Washington, Coulee City	3 Hard white	—	57.8	—	7.1
W-70	Washington, Benge	1 Hard white	—	60.2	—	4.5
W-71	Washington, La Crosse	1 Hard wnite	—	61.0	—	6.0
W-72	Washington, Waitsburg	1 Western red	—	61.5	—	6.8
W-79	Washington, Cashub	1 Western red	—	60.5	—	7.0
W-73	Washington, Garfield	1 White club	—	60.5	—	7.0
W-74	Washington, Dayton	3 White club	—	56.5	—	5.6

[1] Includes both North Dakota and South Dakota.

finest grinding position. After being ground the samples were held in sealed jars in a refrigerator until assayed. All data are given on an "as received" basis, as it was found that conversion to a 15% moisture basis did not change the results other than to lower all values by approximately 0.4 μg. Since the samples were secured during the early part of 1941 it may be assumed that they probably are all from the 1940 crop. There was no correction for a sulfite blank, since it was found that such a correction would be so small as to be within the experimental error.

One hundred and forty-nine samples of wheats with histories indicating origin in at least 18 states, were obtained. Classified by market types the samples include 85 hard red winter, 15 hard spring, 17 soft red winter, 12 white wheats, 9 durum wheats, 9 mixed red winter (predominantly hard), and 2 western red wheats. The thiamin values and detailed data are presented in Table I.

Discussion

A classification of the wheats according to market types, as in Table II, shows that the "hard" wheats—hard winter, spring, and durum—

averaged 7.1 μg of thiamin per gram. The "soft" wheats—soft winter and white and club wheats from the Pacific northwest—averaged 6.1 μg per gram. This might indicate that climates that produce hard wheats with higher protein content also produce higher thiamin content.

TABLE II

THIAMIN VALUES ACCORDING TO MARKET TYPES

Type of wheat	Number samples included	Average thiamin
		μg/g
Durum	9	7.3
Spring	10	6.5
Soft winter	17	6.2
White wheat, including "club"	13	6.0
Western red	2	6.9
Hard winter	70	7.2

Table I shows more samples from Kansas than any other state, and in most instances the county of origin is shown. A line drawn across the state running north and south and passing through Manhattan will roughly show the division of types for this state. In the eastern third, soft or "mixed" wheats are produced, while west of the line the hard winter wheat is more likely to predominate. Neglecting those samples for which no county was given, wheats from the eastern part of the state averaged 7.0 μg per gram, while those west of the line averaged 7.65 μg per gram. The latter approximates the values for the Colorado samples. The average for all samples from Kansas was 7.4 μg per gram.

There was not a sufficient number of pure-variety samples grown

TABLE III

THIAMIN VALUES BY STATES

State	Type	Number of samples	Average thiamin
			μg/g
Colorado	Hard winter	4	7.7
Dakotas	Spring	8	6.5
	Durum	9	7.3
Illinois	—	4	6.0
Kansas	Tenmarq (all hard winter)	10	7.5
	All (all hard winter)	42	7.4
Nebraska	Hard winter	8	7.6
Missouri	Soft winter	6	5.7
Ohio	Soft winter	7	6.1
Oklahoma	Hard winter	11	6.1
Texas	Hard winter	15	6.7
Washington and Oregon	White	14	6.0

over a wide area to show any varietal trends. Tenmarq and Turkey samples were very near the average for the state in which grown.

Average thiamin values for the midwestern states are given in Table III, where it appears that the states producing hard winter wheat (with the exception of Oklahoma) gave thiamin values of 7 μg per gram or more. The states producing soft red winter or white wheats had thiamin values of 5.7 to 6.1 μg. The 8 samples of Dakota spring wheat had an intermediate value of 6.5 μg. There was a wide variation among samples from any state. The values found ranged from the lowest reported by Schultz, Atkin, and Frey (1941) to 10 μg per gram, which is higher than any reported.

Summary and Conclusions

While no categorial statements about the 1940 American wheat crop can properly be based upon the number of samples assayed in this project, what may be a significant trend is noted. A higher thiamin content is found generally in the types of wheat having a hard vitreous berry, with an accompanying higher protein content than is found in the softer types. Hard wheats averaged 7.1 μg per gram as against 6.1 for the soft wheats.

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THE THIAMIN CONTENT OF CEREAL GRAINS[1]

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(Read at the Annual Meeting, May 1941)

With the increasing recognition of the importance of cereals as dietary sources of thiamin has come the need for a more thorough knowledge of the natural vitamin B_1 content of whole grains. Schultz, Atkin, and Frey (1941) have made valuable contributions by their survey of American cereals and Booth (1940) in England has reported on samples of wheat which were grown in all parts of the world. The latter studies in particular have demonstrated the wide variations which occur.

During the course of our investigations a large number of samples of cereal grains have been available. These included many types and varieties grown in different sections of the country. In some instances they represented several series of the same varieties differing only in the location where they were grown. A study of these has been of particular interest since it suggests the existence of certain factors which influence the vitamin content and account for some of the variations observed.

The Hennessy and Cerecedo modification of the thiochrome method was employed for the analyses and the values recorded represent the average of duplicate determinations which agreed within 5%.

Spring Wheats

Table I gives the results obtained on six varieties of spring wheats grown at four stations in Minnesota. The average for these 24 samples was 2.75 mg per pound. In the individual samples, however, there was a considerable difference, since the Marquis grown at Crookston contained only 2 mg, while the Pilot grown at St. Paul had nearly 3.5 mg per pound or 75% more thiamin. When the averages of the different varieties grown at the same locations are considered it is apparent that the vitamin content varies with the location.

In this series of spring wheats the samples from the St. Paul station had the highest values and those from Crookston the lowest. Those from Morris and Waseca occupied intermediate positions. On the other hand, the averages for single varieties grown at the different locations did not show material differences. With the exception of Thatcher all are within practically 5% of the average.

These observations indicate that for these particular samples, at least,

[1] Paper No. 30, Journal Series, General Mills, Inc., Research Laboratories.

TABLE I

THE THIAMIN CONTENT OF SPRING WHEATS
(Thiamin in milligrams per pound—13.5% moisture basis)

Variety	Location grown (Minnesota)				Average
	St. Paul	Morris	Waseca	Crookston	
Renown	3.12	3.15	2.61	2.32	2.80
Rival	3.15	2.85	2.76	2.41	2.80
Pilot	3.49	2.75	2.94	2.42	2.90
Thatcher	3.08	2.63	2.39	2.10	2.55
Ceres	3.28	3.18	2.68	2.17	2.83
Marquis	2.82	2.96	2.71	2.01	2.62
Average	3.16	2.92	2.68	2.24	2.75

environmental factors play a large role in deciding the thiamin content of wheat. Varietal factors are of much less consequence.

Table II gives the protein values of these same wheat samples. Any trends in protein did not seem to follow those found for the vitamin.

TABLE II

THE PROTEIN CONTENT OF SPRING WHEATS
(Protein in percent—13.5% moisture basis)

Variety	Location grown (Minnesota)				Average
	St. Paul	Morris	Waseca	Crookston	
Renown	16.9	16.6	15.7	15.7	16.22
Rival	15.4	14.8	14.4	14.8	14.85
Pilot	15.5	14.1	14.4	15.3	14.82
Thatcher	15.8	16.2	15.1	16.1	15.80
Ceres	15.8	15.4	14.1	15.6	15.22
Marquis	15.8	15.8	13.6	15.6	15.20
Average	15.87	15.48	14.55	15.52	15.35

Thus at Crookston where the samples were lowest in thiamin the average protein was similar to that of the Morris-grown samples. Similarly, with the averages from the single varieties no relation to thiamin appears.

Table III shows the values for ash. There was a definite relationship between this constituent and thiamin, since the samples from locations where the ash contents were highest and lowest were also similarly related in vitamin content.

These data have been analyzed statistically. There is no significant relation between thiamin and protein, the correlation coefficient being + .127. With ash, however, the correlation is highly significant. Figure 1 shows this relation. The correlation coefficient is + .680 and the 1% point is + .515 for the 24 pairs. This means that the chances are

TABLE III

THE ASH CONTENT OF SPRING WHEATS
(Ash in percent—13.5% moisture basis)

Variety	Location grown (Minnesota)				Average
	St. Paul	Morris	Waseca	Crookston	
Renown	1.99	2.09	1.97	1.94	1.997
Rival	2.04	1.69	1.92	1.77	1.855
Pilot	2.11	1.67	1.88	1.63	1.822
Thatcher	1.92	1.91	1.89	1.78	1.874
Ceres	2.28	2.08	2.00	1.70	2.015
Marquis	2.11	2.00	1.97	1.78	1.965
Average	2.075	1.907	1.938	1.767	1.922

better than 100 to 1 that the ash content is a rough index of the vitamin content. The equation which best fits the data is shown with the plot of the individual values.

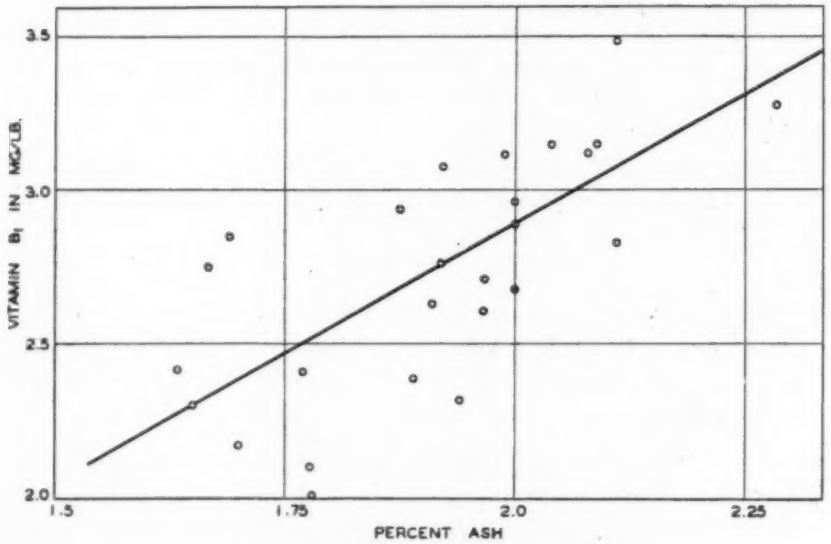

Fig. 1. Relationship between thiamin and ash.

Variance analysis of the thiamin content observed in the different varieties grown at the several locations has also been made. It appears that the effect of location is approximately 15 times greater than that due to variety. The effect of location is highly significant, while that of the variety is not appreciably greater than the experimental error.

While the number of samples examined was too small to justify definite conclusions it appears that environment is an important element in

deciding the thiamin content and that the same environmental factors which influence ash may also influence thiamin.

Winter Wheats

It should not be assumed, however, that varietal factors are entirely without effect. Table IV shows some results obtained on winter wheat varieties where environmental conditions alone did not account for the variations.

TABLE IV

THE THIAMIN CONTENT OF WINTER WHEATS
(Thiamin in milligrams per pound—13.5% moisture basis)

Variety	Location grown (Nebraska and Kansas)				Average
	Lincoln	Dodge City	Wichita	Hutchinson	
Chiefkan	2.44	2.25	1.82	1.68	2.05
Blackhull	2.24	2.30	2.01	1.98	2.13
Turkey	2.48	2.57	2.01	2.05	2.28
Tenmarq	2.69	2.70	2.50	2.27	2.54
Average	2.46	2.45	2.09	2.00	2.25

While there was a trend to lower values in the samples from Wichita and Hutchinson, there was also a trend to different values in the varieties. Thus Tenmarq and Turkey, of the four varieties studied, were higher than Chiefkan and Blackhull grown at the various stations. Variance analysis of the data shows that both variety and environment are highly significant and are of similar magnitude.

An examination of the ash content of these winter wheats failed to show the high correlation between thiamin and ash observed in the spring-wheat samples.

TABLE V

THE ASH CONTENT OF WINTER WHEATS
(Ash in percent—13.5% moisture basis)

Variety	Location grown (Nebraska and Kansas)				Average
	Lincoln	Dodge City	Wichita	Hutchinson	
Chiefkan	1.53	1.76	1.45	1.53	1.57
Blackhull	1.47	1.89	1.61	1.78	1.69
Turkey	1.69	1.80	1.76	1.81	1.77
Tenmarq	1.56	1.97	1.51	1.73	1.69
Average	1.56	1.85	1.58	1.71	1.68

The ash values given in Table V are the highest for the samples grown at Dodge City. The samples were also high in thiamin. On the other hand, the Lincoln-grown samples which contained very similar quantities of thiamin had the lowest average amount of ash. Nor is the varietal effect exhibited in vitamin content reflected in the ash values. A statistical analysis of the data shows the correlation coefficient to be + .2747 and the 5% point + .497.

This absence of any significant relation between thiamin and ash is in agreement with the observations on a large number of Canadian hard red spring wheats reported by Johannson and Rich (1941). Obviously the relation found in the spring wheats is not representative for all varieties and all regions. It has been suggested that some single mineral constituent may be closely related to thiamin but that the relation between such a constituent and total ash may vary widely. Only where total ash reflects the content of this unknown mineral will a relation between thiamin and ash possibly appear.

The iron content of the winter wheats listed in Tables IV and V has been determined. Table VI gives the values for this element.

TABLE VI

THE IRON CONTENT OF WINTER WHEATS
(Iron in micrograms per gram—13.5% moisture basis)

Variety	Location grown (Nebraska and Kansas)				Average
	Lincoln	Dodge City	Wichita	Hutchinson	
Chiefkan	49	45	44	40	44.5
Blackhull	44	42	45	37	42.0
Turkey	47	43	57	38	46.2
Tenmarq	43	40	41	34	39.5
Average	45.8	42.5	46.8	37.2	43.1

The average iron values for the Hutchinson-grown samples was lowest in the series, a rating also observed for thiamin. However, the Wichita samples which were very similar to those from Hutchinson in thiamin content exhibited the highest average for iron. Similarly, there is no apparent relation between vitamin B_1 and iron in the averages for the different varieties. The correlation coefficient for the sixteen pairs is — .020, an even lower value than that found for ash. It would appear that iron is not the unknown mineral, if such exists, which is related to vitamin B_1 content.

Table VII presents the thiamin values obtained on a number of different winter wheats. These are miscellaneous varieties grown in vari-

ous parts of the winter wheat area. As in the other winter wheat samples Turkey and Tenmarq are appreciably higher in thiamin than Chiefkan and Blackhull. Also, the average of all 19 samples and the range between the low and high values are very similar to those of the previous series. These samples were not grown at the same locations and because of the effect of environment it can be anticipated that the relative values might be different if collections were made from other regions. Extensive studies over several crop years will be required to establish varietal superiorities from the standpoint of thiamin content.

TABLE VII

THIAMIN CONTENT OF MISCELLANEOUS WINTER WHEATS
(Thiamin in milligrams per pound—13.5% moisture basis)

Variety	Source	Thiamin
Chiefkan	Falun, Kansas	1.78
Chiefkan	Guthrie, Okla.	1.67
Karmont	Billings, Mont.	1.73
Iobred	Ames, Iowa	2.00
Kanred	Bird City, Kans.	2.41
Blackhull	Claude, Texas	1.65
Superhard Blackhull	Eagle City, Okla.	1.99
Superhard Blackhull	Meade, Kans.	1.85
Turkey	Manhattan, Kans.	2.27
Turkey	Manhattan, Kans.	2.18
Turkey	Meno, Okla.	2.56
Nebred	Manhattan, Kans.	2.08
Cheyenne	Manhattan, Kans.	2.27
Tenmarq	Preston, Kans.	2.08
Tenmarq	Manhattan, Kans.	2.70
Tenmarq	Orienta, Okla.	2.47
Kawvale	Manhattan, Kans.	1.98
Kawvale	Lincoln, Nebr.	2.71
Kawvale	Talala, Okla.	2.03
Average		2.12

Soft Wheats

Soft wheats have also been included in our survey and the results are shown in Table VIII. These samples were all grown at the same location in Ohio. It is interesting to note the constancy of the vitamin values for the different varieties. Twelve of the 15 samples were within 3% of the average and may be considered to be identical. Two of the remaining three were only about 10% below the average, leaving the last variety, Canawa, as somewhat higher in thiamin content. Further studies would be necessary to determine whether this is a true varietal effect since only single samples are represented.

TABLE VIII

VITAMIN B_1 CONTENT OF SOFT WHEATS (OHIO)
(Vitamin B_1 in milligrams per pound—13.5% moisture basis)

Variety	Vitamin B_1
Trumbull	2.03
Purdue	1.99
Dawson	2.03
Fulcaster	2.10
Bald Rock	1.79
Wabash	1.87
Red Rock	2.02
Fulhio	2.09
Poole	2.02
Fultz	2.05
Gladden	1.97
Thorne	2.05
American Banner	2.01
Yorkwin	2.05
Canawa	2.38
Average	2.03

West Coast Wheats

Table IX shows the values obtained on a number of wheats grown on the west coast. They represent several different types and varieties and have an average thiamin content similar to that of the winter wheats grown in the midwestern states. The range of values is also very similar.

TABLE IX

VITAMIN B_1 CONTENT OF WEST COAST WHEATS
(Vitamin B_1 in milligrams per pound—13.5% moisture basis)

Variety	Vitamin B_1
Baart	2.31
Baart No. 17	2.24
Baart No. 38	2.11
Federation	1.76
Kharkof	1.94
Brevon	1.77
Turkey	1.85
Blue Stem	2.44
Ridit	1.97
Rex	2.28
Hymar	2.36
Oro	2.04
Average	2.09

Canadian Wheats

Table X gives the thiamin values found in several varieties of Canadian wheats. Each variety was grown at Winnipeg and at one

other Canadian point. In all cases the Winnipeg samples were higher in vitamin content than the corresponding varieties grown elsewhere. This is another illustration of the profound effect of environment. Two of the varieties, Thatcher and Renown, were common to the Minnesota samples shown in the first table. The values for the Winnipeg samples were similar to those grown in St. Paul, while those grown at the other Canadian stations were almost identical with the Crookston samples.

TABLE X

VITAMIN B_1 CONTENT OF CANADIAN WHEATS

(Vitamin B_1 in milligrams per pound—13.5% moisture basis)

Variety	Source	Vitamin B_1	Ash
			%
Red Bobs	Winnipeg	2.70	1.44
Red Bobs	Airdrie, Alta.	1.84	1.11
Thatcher	Winnipeg	2.64	1.81
Thatcher	Saltcoats, Sask.	2.10	1.45
Apex	Winnipeg	2.38	1.66
Apex	Kelvington, Sask.	1.99	1.45
Garnet	Winnipeg	2.28	1.48
Garnet	Lacombe, Alta.	1.45	1.62
Reward	Winnipeg	2.61	1.53
Reward	Coderre, Sask.	2.35	1.50
Renown	Winnipeg	2.81	1.79
Renown	Gilbert Plains, Man.	2.24	1.33
Mindum	Winnipeg	2.69	1.47
Average of Winnipeg samples		2.59	1.60
Average of other samples		1.99	1.41
Average of total samples		2.31	1.51

The samples include one variety of durum wheat, Mindum, the thiamin content of which was about the same as the average for the spring wheats. A similar observation was made on two durum samples grown in St. Paul. These were similar to spring wheats from the same location.

The sample of Garnet wheat grown at Lacombe contained the lowest amount of thiamin which we have observed. This is nearly twice as high, however, as the 0.5–0.6 International Unit reported by Booth (1940) for samples of Manitoba No. 3 and No. 1 Dark Northern Spring.

The ash content of these Canadian wheats has also been determined and the values are included in the table. There was no significant correlation between thiamin and ash, the correlation coefficient being + .402 and the 5% point, + .553. It was interesting to note that, with the exception of the Garnet variety, the Winnipeg-grown samples were higher in both thiamin and ash than the corresponding varieties grown at the other locations. Despite this there was no significant relation between the vitamin and ash in either group.

Other Cereal Grains

Table XI summarizes the averages for the various wheats examined and compares these values with those of other cereal grains. With the exception of oats, these other grains were similar to wheat in thiamin content. While at least six varieties of each were studied the number of locations where they were grown was too limited to justify the acceptance of their values as representative averages. The oats, barleys, and many of the corns were obtained from stations which showed a high vitamin content in wheats. For this reason nationwide averages might be expected to be somewhat lower.

TABLE XI

THIAMIN CONTENT OF CEREAL GRAINS
(Thiamin in milligrams per pound)

Grain	Number of samples	Vitamin B_1	
		Average	Range
Spring wheats			
Minnesota	24	2.75	2.01–3.49
Canada	13	2.31	1.45–2.81
Winter wheats	35	2.17	1.65–2.71
West Coast wheats	12	2.09	1.76–2.44
Soft wheats	15	2.03	1.79–2.38
Corn			
Yellow	6	2.44	2.19–2.82
White	6	2.76	2.24–3.04
Oats	6	4.20	3.68–4.90
Rye	6	2.11	1.88–2.28
Barley	6	2.95	2.58–3.33
Grain sorghums	7	2.68	1.93–3.97

Summary

The evidence presented indicates that the thiamin content of wheat is influenced by the wheat type, variety, and environment. In general, durum and spring wheats contained the largest amount of vitamin B_1, followed by hard winter and soft wheats in this order. A significant effect of variety was observed in a series of winter wheats. Environment is an important factor, since the same varieties grown at different locations differed widely in content of thiamin.

In a series of spring wheats a high correlation between thiamin and ash was observed. This relation did not prevail in the instance of other spring wheats or a series of winter wheat varieties. Corn, rye, barley, and sorghum were found similar to wheat in their thiamin contents. Oats contained somewhat more of this vitamin.

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THIAMIN IN THE PRODUCTS OF WHEAT MILLING AND IN BREAD[1]

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(Read at the Annual Meeting, May 1941)

Of the many vitamins known to be necessary for man, wheat contains several members of the water-soluble vitamin B complex, thiamin, riboflavin, nicotinic acid, vitamin B_6, and pantothenic acid. It also contains the fat-soluble vitamin E. Thiamin (vitamin B_1) is very important from the viewpoint of flour millers since wheat is regarded as an excellent source of this vitamin.

If the estimate of 6½ ounces is accepted as the average daily per capita consumption of flour, and if it is assumed for illustration that it is all whole-wheat flour, the thiamin contribution to the daily diet will be about 0.9 mg. This is one-half the daily requirements of a moderately active man according to figures recommended by the Committee on Food and Nutrition of the National Research Council (1941).

Wheat flour has not been contributing this amount of thiamin to the human dietary in the past, however, as only a small portion of the flour consumed in this country is actually made from the entire grain. There-

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fore, any practical evaluation of wheat for its nutritional service to man must be based upon commercial types of white flour rather than upon whole-wheat flour.

The Distribution of Thiamin in Products of Milling

As a whole wheat is recognized as an excellent source of thiamin, it is important to examine the wheat kernel and the products of milling to determine the distribution of this vitamin. The distribution is of special interest in view of the current program of enriched flour production.

The vitamins and minerals of wheat are not uniformly distributed throughout the kernel, but are highly concentrated in certain tissues, while others contain only small amounts. The outer layers and the germ are high in these factors, the endosperm relatively low. Patent flour, comprised almost entirely of endosperm, has about one-seventh of the thiamin concentration of whole-wheat flour. Of the total thiamin in the wheat kernel, the endosperm contains about 24%, the embryo about 15%, and the outer layers of the kernel about 61%.

The values just cited are not based on milling yields but on calculations which show the thiamin distribution between endosperm, embryo, and bran. They do not give a true picture of the thiamin content of the products of milling, since it is impossible to make a sharp separation between the different structures in the wheat kernel in the commercial process of milling.

Analyses of products of commercial wheat milling have been made to determine the distribution of thiamin in certain well-known commercial products. The thiamin content of the products from three different hard-wheat mixes is shown in Table I. These values are in good agreement with those reported by Schultz, Atkin, and Frey (1939), considering the variability in wheat and in milling practices.

TABLE I

THIAMIN (VITAMIN B_1) CONTENT OF PRODUCTS OF COMMERCIAL WHEAT MILLING
(Samples taken from three different hard wheat mixes)

Sample	Thiamin (mg per pound)		
	Mix 1	Mix 2	Mix 3
Cleaned wheat	2.18	2.35	2.31
Patent flour	0.29	0.29	0.35
First clear flour	1.28	1.31	1.50
Second clear flour	6.22	6.05	4.57
Red dog flour	13.60	14.42	12.32
Germ	10.65	10.20	—
Shorts	4.31	9.07	10.28
Bran	4.04	3.73	4.98

The wheat mixes were similar in thiamin content, two of them being practically identical. The patent flours are also similar as are the first clear and red dog. There is greater variation in the other products of milling from different wheat mixes, especially the shorts. Variations in milling practice can account for large differences in thiamin content of these materials. By taking average values for the milling yields, the percent of the total wheat thiamin in each of these mill products has been calculated. These values are shown in Table II.

TABLE II

THIAMIN (VITAMIN B_1) DISTRIBUTION IN PRODUCTS OF COMMERCIAL WHEAT MILLING

(Averages of samples from several millings)

Sample	Milling yield	Thiamin content	
	Percent of cleaned wheat (approximate)	Mg per pound	Calculated as percent of total thiamin in wheat
Patent flour	63.0	0.31	8.0
First clear flour	7.0	1.36	3.9
Second clear flour	4.5	5.61	10.0
Red dog flour	4.0	13.45	22.0
Germ	0.2	10.40	0.9
Shorts	12.3	7.89	39.6
Bran	9.0	4.25	15.6
Cleaned wheat	100.0	2.28	100.0

Patent flour, representing 63% of the cleaned wheat, contains only 8% of the total thiamin of the whole grain, whereas second clear flour, representing only 4.5% of the wheat, contains 10% of the thiamin. Red dog flour contains 22% of the thiamin.

The percent of thiamin represented by germ appears to contradict the previous statement respecting the amount of thiamin in the embryo. This apparent discrepancy is due to the fact that commercial milling does not permit the recovery of all the germ contained in the wheat. In the case of the samples shown in Table II, less than 10% of the total germ was recovered in the germ stream. The other 90% is in the feeds.

The figures presented in Table I were derived from the products of two mills. Since milling practices differ in different mills the data cannot be regarded as representative of commercial milling operations. Accordingly, our studies were extended to include a wider variety of products. The results shown in Table III were obtained on samples from nine different mills. Ash values are also shown to indicate the approximate degree of extraction.

TABLE III

THIAMIN AND ASH IN PRODUCTS OF COMMERCIAL WHEAT MILLING
(Includes products from nine mills)

Sample	Thiamin (mg per lb) [1]		Ash (%)	
	Average	Range	Average	Range
Cleaned wheat	2.20	1.97– 2.43	1.56	1.40–1.75
Patent flour	0.32	0.23– 0.48	0.40	0.36–0.43
First clear	1.20	0.50– 1.66	0.68	0.44–0.85
Second clear	3.76	2.00– 5.34	1.33	0.88–2.34
Red dog	11.74	10.39–13.45	2.83	2.44–3.70
Shorts	8.03	5.60– 9.83	4.35	3.63–5.16
Bran	3.99	2.76– 4.98	6.52	6.30–6.94

[1] All samples contained about 11% moisture at time of analysis.

In general the average values for thiamin are in good agreement with those obtained from the products of a single mill (Table I). There is, however, a rather wide range in the vitamin content of each of the different grades of flours and feeds. This cannot be attributed to the amount of thiamin present in the individual wheats since all are within 10% of the average.

Since ash values reflect to a considerable extent the types of milling, it is of interest to compare these values with those for thiamin. Such a comparison is presented in Figure 1.

There is a close parallelism between thiamin and ash in the patent,

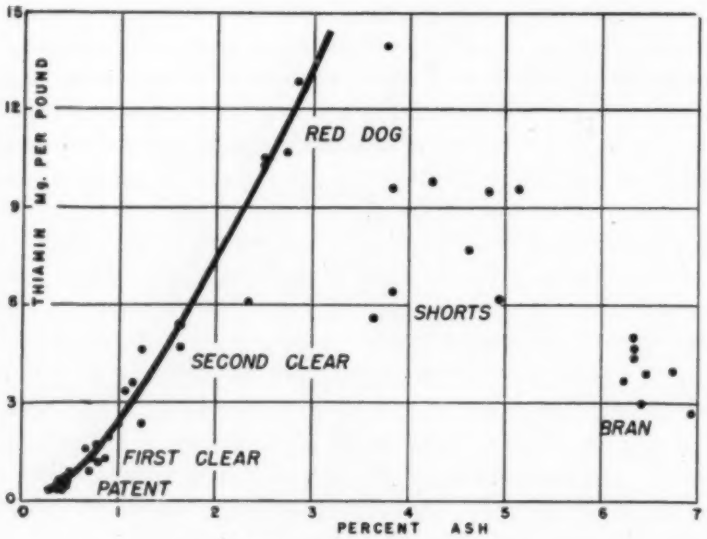

Fig. 1. Relation of thiamin to ash in products of wheat milling.

clear, and the red dog flours. In the shorts and bran, however, these relations disappear and the further increase in ash shows a marked and irregular decrease in thiamin. This can be in part explained by the relatively low thiamin content of bran, a considerable amount of which is present in the shorts.

To show the relationship between long extraction and thiamin content, the values given in Table II have been calculated differently in Table IV.

TABLE IV

RELATION OF PERCENT OF EXTRACTION TO PERCENT OF TOTAL THIAMIN IN CLEANED WHEAT

(Theoretical values obtained by calculation, not by actual production of long extraction flour)

Sample	Extraction—percent of cleaned wheat	Percent of total thiamin in cleaned wheat
1. Patent flour	63.0	7.8
2. Patent flour plus first clear	70.0	11.8
3. (2) plus second clear	74.5	23.8
4. (3) plus red dog	78.5	48.8
5. (4) plus germ and shorts	91.0	85.4
6. (5) plus bran (entire wheat)	100.0	100.0

It will be noted that an addition of 7% of first clear to patent flour raises the thiamin content of the resultant straight-grade flour to 50% above the patent. A further addition of 4.5% of second clear doubles the thiamin content of the straight grade. Another addition of 4%, in the form of red dog, again doubles the thiamin content. It must be pointed out that the thiamin percentages shown in this table for the long-extraction products were obtained by calculation and not by actual milling to such high extractions. The same values are shown to better advantage in a curve (Fig. 2) where percent of total wheat is shown on the horizontal axis and percent of total thiamin in wheat on the vertical axis. Below 70% extraction the curve rises gradually, while a sharp rise occurs between 73% and 79%. Above 80 the slope again changes, and as the curve approaches 100 each increment of increase in extraction brings less increase in thiamin. This is due to the relatively low thiamin content of the outer bran layer.

Our values and those reported by others show clearly that the more refined flours are lower in thiamin. Since ash is a criterion of refinement, it seemed desirable to determine the correlation between the two factors. Hoffman, Schweitzer, and Dalby (1940) have previously noted a relationship between ash and thiamin content.

In the course of our investigations to determine the distribution of thiamin in the products of wheat milling a large number of mill streams

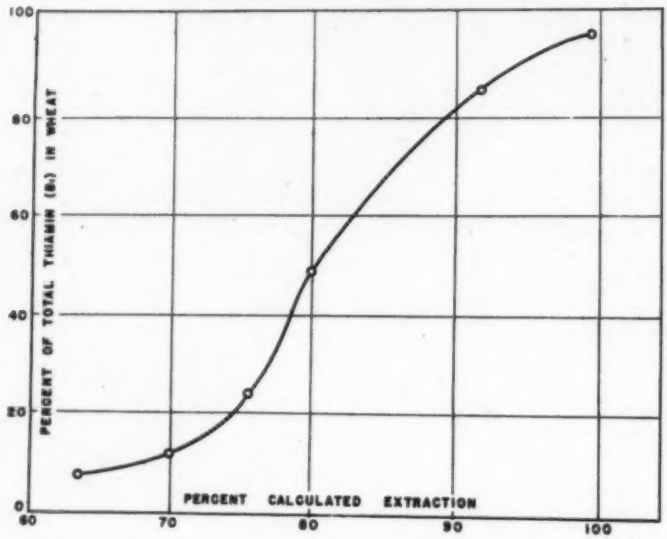

Fig. 2. Relation of thiamin to percent extraction of wheat.

have been examined in addition to commercial products of milling. These mill streams have been obtained from mills all over the United States and, accordingly, represent a variety of milling operations performed on many types of domestic bread wheats. The ash contents of the streams range from the low values of patent flour to the high values of low-grade flours and feeds. A high positive correlation has been found between thiamin and ash contents. Figure 3 shows the relationship graphically.

For the entire population the coefficient is $r = +.9498$ indicating a very high correlation. When three bran duster flours high in ash and relatively low in thiamin are omitted, the correlation becomes $r = +.9716$ for the curves shown in Figure 3. The solid line represents all samples, the dotted line all except the three bran duster streams. Statistically there is a significant difference between these two correlations and hence in the calculation of r there is justification for the omission of the three samples noted above, since it is clear that they belong in a separate classification. Failure of the three bran duster samples to fall in line with the other mill streams and the fact that the curve flattens in the high ash range show clearly that in practical mill operations ash content cannot be used as the sole criterion for selecting high vitamin B_1 mill streams unless it is known that the streams are not rich in bran tissue.

The high correlation between ash and thiamin prevails until the ash content reaches about 2.5%. With higher ash the thiamin increase is

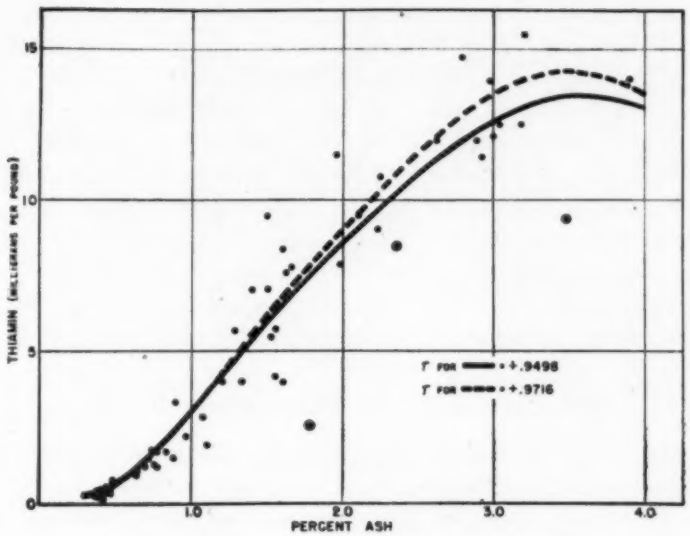

Fig. 3. Relation of thiamin to ash in mill streams.

somewhat less. Reference to Table II shows that bran contains less thiamin than second clear, red dog, germ, and shorts. This is due to the relatively low thiamin content of the outer bran or pericarp. Since bran is very high in ash it is to be expected that streams rich in bran tissue will not show the same relation between thiamin and ash as do the other streams.

The data show clearly that much of the thiamin in whole wheat is contained in the fractions of milling which normally go into the animal feeds. Low-grade or red dog flour is especially high in thiamin content, exceeding germ in the samples tested in this study. This was especially interesting, as germ has been frequently reported as an unusually rich source of thiamin.

Microscopic examination of the low-grade flours separated in the laboratory did not reveal the predominance of any specific tissue of the wheat kernel. Aleurone and branny layers and starch cells were identified. The outer bran layer (pericarp) could not have been present in significant amount, since actual tests of purified bran have shown that it is not rich in thiamin.

These observations have led us to postulate that the distribution of thiamin in wheat may be due largely to the conditions which prevail during the period of growth when the kernel is maturing. During the milk stage the kernel has a high water content, and since thiamin is water-soluble, it is conceivable that the distribution *may* be more uniform at that time, although evidence is not now available to support this

speculation. As the kernel ripens, water moves from the inside to the surface and is lost. It is believed that this movement of water may result in translocation of the vitamin to the layers of the kernel surrounding the endosperm, where it becomes concentrated as the water evaporates. Thiamin does not become concentrated, however, in the outer bran layer, or pericarp, and tests of pure bran do not show high thiamin. This suggested explanation respecting disposition of thiamin might account for failure to find high vitamin concentration in the outer tissue. Concentration of thiamin in the layers of tissue just underneath the pericarp would also probably account for the rapid increase in thiamin when milling extraction is increased from 73% to 79%.

Thiamin Content of Whole Wheat Bread

In addition to the examination of several mill products, whole-wheat flours have been tested for thiamin. Thirty-one samples of commercial whole-wheat flours collected from bread-wheat mills distributed about the United States were found to contain an average of 2.27 mg of thiamin per pound. The values ranged from 1.92 to 2.53, a much narrower range than found by Nordgren and Andrews (1941) in samples of whole wheat obtained from experimental plots. The latter ranged from 1.4 to 3.2. The blending of large lots of commercial wheats in making wheat mixes for grinding into whole-wheat flour is undoubtedly the explanation of the smaller variations in thiamin in commercial whole wheat flours.

Each of the 31 samples of commercial whole-wheat flours was baked in the laboratory with a commercial formula. The whole-wheat bread averaged 1.36 mg of thiamin per pound of fresh bread. The relationship between thiamin in flour and thiamin in fresh bread was quite uniform in comparisons of individual samples of whole-wheat flour and bread. The concentration of thiamin in the fresh bread containing 38% moisture averaged 60% of that in the flour, the individual samples ranging from 58% to 61%. This does not, of course, mean that 40% of the thiamin is lost during baking, since the lower concentration in the bread is due in a large degree to the dilution of the vitamin by the water in the bread. Actual vitamin losses were not calculated since the thiamin content of the ingredients other than flour was not determined. The study was made in order to evaluate approximately the thiamin content of commercial whole-wheat loaves.

During the course of our investigations two samples of foreign "war flours" were made available. A sample from Switzerland contained 0.87 mg per pound, and one from Italy contained 1.14 mg per pound.

Summary

The distribution of thiamin in wheat as determined by the analysis of the products of commercial milling is reported and discussed. The major part of the vitamin occurs in those tissues of the wheat kernel just beneath the outer bran. These tissues are responsible for the high thiamin content of the feeds such as red dog and shorts. A possible explanation for this distribution is presented.

There is a high correlation between thiamin and ash in the milling fractions having 2.5% or less of ash. Above this value the correlation is less significant because of the varying amounts of outer bran tissues, which are low in thiamin.

The thiamin contents of whole-wheat flours and breads prepared therefrom are reported.

Acknowledgments

The authors are indebted to the Products Control Division of General Mills, Inc., for the samples and ash analyses reported in this paper.

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THE IRON CONTENT OF CEREALS[1]

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(Read at the Annual Meeting, May 1941)

The iron content of whole cereal grains, especially wheat, has long been recognized as a property of considerable nutritional value. Medical authorities have advocated consumption of whole-wheat products as a means of increasing the amount of iron supplied by the average human dietary.

Since much of the iron in wheat is distributed throughout the tissues adjacent to and constituting the bran layer, the milling of flour yields a product that is relatively low in this nutritive element. Cognizance of this has been taken by those agencies interested in establishing

[1] Paper No. 35, Journal Series, General Mills, Inc., Research Laboratories.

standards for "enriched" flour and bread, with the result that the addition of an assimilable iron compound has been made a prerequisite for these recently proposed products.

This action has introduced a new problem for many of the laboratories serving the flour manufacturer and allied trades. In the past the absence of any great demand for analyses of iron in cereal products has confined such activities to a few laboratories interested in problems of a research nature. From this status the demand has suddenly increased so that today methods of analysis are of interest to many other laboratories, notably those engaged in the control of manufactured cereal products.

Discussion of Analytical Methods for Iron

An examination of the literature reveals a rather confusing mass of information on the subject of the iron analysis of foods. Numerous observations are reported about the difficulties which attend the use of various methods and many modifications have been proposed for the elimination of factors that adversely affect the successful operation of these methods. In several instances the modifications have so greatly complicated the procedure that the analyst must be painstaking and expert to avoid the introduction of serious errors. With this in mind the writers have examined some of the problems involved, especially as they pertain to cereals and cereal products.

The widespread use of colorimetric methods is tacit acknowledgment of the inadequacies of the usual volumetric and gravimetric methods. In most instances the amount of iron contained in cereals is too low to permit convenient application of the latter procedures. Thus, one finds an almost universal employment of colorimetric techniques, which are better adapted to the analysis of relatively minute quantities. Many substances that produce colored compounds with iron have been used. Among these are ferricyanide and sulfocyanate in the inorganic, and *o*-phenanthroline, mercaptoacetic acid, and α,α'-dipyridyl in the organic fields. Of these substances, the sulfocyanate has been most extensively studied and widely used, and only recently has α,α'-dipyridyl been employed outside its special application to the determination of "available iron" in foods.

The use of either of these reagents requires that the iron compound under examination be present in a reactive form. With cereals it must be contained in a solution free from substances which inhibit the development of color when treated with the reagent. Prominent among the inhibitors is pyrophosphate, which forms during the dry ashing of the sample. In the presence of pyrophosphate iron does not react completely with sulfocyanate and a quantitative evaluation of the iron is prevented.

This inhibitory effect can be eliminated by employing so-called "wet ashing" procedures, which prevent the formation of pyrophosphate, or by hydrolysis of the solution of ash prior to the addition of the reagent. Hydrolysis can be effected by heating either with acids or alkali. Elvehjem (1930) proposed the use of alkali for samples having a high pyrophosphate content while Farrar (1935) and Shackleton and McCance (1936) employed hydrochloric acid. Stugart (1931) expressed a preference for the acid method and pointed out that the use of alkali introduced variable quantities of foreign iron compounds. Hoffman, Schweitzer, and Dalby (1940) used electrolytic caustic to avoid iron contamination. In a recent study of the α,α'-dipyridyl method Jackson (1938) eliminated pyrophosphate interference with hydrochloric acid but apparently found that hydrolysis was slow since the ash solution was heated at 80°C for 18 hours.

A standard method for the hydrolysis of pyrophosphates in analytical practice is fusion with sodium carbonate. Since this compound can be readily purified by crystallization from the bicarbonate, this method offers a means of removing pyrophosphate from cereal ash. The writers have studied this possibility by mixing the ash with the carbonate and fusing the mixture in a platinum crucible. Two minutes' fusion was adequate to destroy any pyrophosphate as evidenced by instant and complete development of color with dipyridyl. No serious contamination with iron was found when the same procedure was applied without the ash.

From the standpoint of convenience, however, the fusion with carbonate is not the most desirable since the fusion vessels must be kept scrupulously clean and a quantitative transfer of the ash from the ashing dish is required. Also, the ash should be thoroughly mixed with the carbonate, preferably by grinding, and this step requires considerable care. With the proper technique, however, excellent results can be obtained.

The use of acid hydrolysis offers by far the simplest method. Solution of the ash can be effected directly from the ashing crucible and a few minutes' heating is all that is required to destroy interfering salts.

For developing the color of the iron α,α'-dipyridyl has been chosen since it is convenient to use and the color is stable and easily measured. The iron solution is first adjusted to the proper pH by the addition of a buffer solution and any ferric iron is then reduced by adding a solution of hydroquinone. The dipyridyl solution is then added and the color measured. In order to compensate for any traces of iron which may be present in the various reagents a blank is prepared and this solution is used for adjusting the photoelectric colorimeter.

In addition to the interference of pyrophosphates a second factor reputed to cause analytical errors has been given considerable attention in the literature. This concerns the loss of iron by volatilization during the ashing process. Jackson (1938) reported that iron losses can be avoided only by "wet ashing" with a mixture of nitric, sulfuric, and perchloric acids. Hoffman, Schweitzer, and Dalby (1940) moistened samples of bread with alkali prior to ashing and thereby prevented losses which otherwise were very large. Farrar (1935) ashed samples with calcium carbonate to avoid contamination by the acids used in "wet ashing." He found, however, that losses of iron during the usual dry ashing were not always evident since the analysis of a sample of dog bread gave the same result when carbonate was used and when it was omitted.

For the cereal chemist it is of considerable advantage to be able to analyze the ash obtained from the regular ash determination. Accordingly, the writers have investigated the loss of iron as evidenced by the recovery of added iron compounds. These have included ferric chloride since losses are generally attributed to the formation of this somewhat volatile iron salt. The results are shown in Table I.

TABLE I

THE RECOVERY OF ADDED IRON IN THE ANALYSIS OF FLOUR

Sample	Added iron	Calculated	Found
	%	%	%
Flour	none	—	0.0009
Flour plus iron pyrophosphate	0.0060	0.0069	0.0071
Flour plus iron phytate	0.0035	0.0044	0.0042
Flour plus reduced iron	0.0035	0.0044	0.0042
Flour plus iron mucate	0.0035	0.0044	0.0044
Flour plus ferric chloride	0.0035	0.0044	0.0045

These analyses were made by ashing overnight the samples at about 575°, heating the ash with a few milliliters of dilute hydrochloric acid, making to volume, and measuring the color developed by dipyridyl in an aliquot of the solution. There is no evidence of any loss of iron since the differences between the found and calculated values are within the experimental error of the method.

Similar excellent recoveries have been obtained from the analyses of breads made from flour containing added iron. These experiments included one series of breads made with varying amounts of salt, an ingredient which might promote a loss of iron during ashing. Table II presents the results.

There seems to be no evidence that normal amounts of salt cause losses of iron under the analytical conditions employed.

TABLE II

EFFECT OF SALT ON THE IRON ANALYSIS OF BREAD

Salt in bread	Iron
%	%
0	0.0122
1	0.0122
2	0.0119
4	0.0121

Iron Content of Wheat and Its Products of Milling

Using the dry ashing procedure and the dipyridyl method, samples of various wheats and mill products have been examined. Wheats normally contain between 30 and 50 μg of iron per gram although values as low as 27 and as high as 60 have been obtained on a few samples. Most bread flours of patent grade range between 6 and 9 μg per gram or 2.7 to 4.1 mg per pound. Occasionally iron values as low as 4 μg per gram and as high as 12 have been observed but these appear to be unusual.

The other products of milling vary widely in their content of iron. Table III shows the results found by the examination of all the products obtained from a single commercial milling of wheat.

TABLE III

THE IRON CONTENT OF THE PRODUCTS OF WHEAT MILLING

(Basis 13.5% moisture)

Product	Ash	Iron	Iron in ash
	%	%	%
Patent flour	0.41	0.00078	0.190
First clear	0.82	0.0019	0.232
Second clear	2.34	0.0062	0.265
Red dog	3.70	0.0113	0.306
Shorts	4.18	0.0127	0.305
Bran	6.53	0.0126	0.193
Germ	4.14	0.0086	0.208
Wheat	1.65	0.0037	0.224

While in most instances there is an increase in the iron content as the ash increases, this relation does not hold for all the mill products. Thus, bran with nearly 50% more ash than shorts actually contains about the same amount of iron. Similarly, germ is considerably lower in iron than shorts although its ash content is approximately the same. This suggests that the mechanism responsible for the distribution of iron in the wheat berry may differ with respect to the other mineral constituents. It demonstrates that the ash content does not serve as an accurate index of the quantity of iron present. This was also

observed in the case of patent flours where widely varying amounts of iron were found in samples of similar ash content. The last column in the table shows how the ratio of iron to ash varied in the particular products examined. The ratio is lowest in the patent flour and bran and highest in red dog and shorts. Wheat occupies an intermediate position, as of course it should, to account for the ratios in its mill products. On the basis of approximate mill yields an average ratio can be calculated for the different fractions in the proportion of their amounts in wheat. This calculated value is 0.222, which agrees well with the observed value of 0.224.

These findings are not entirely in accord with the results reported by Sullivan and Near (1927), who analyzed a series of mill products and found a rather constant relationship between ash and iron. In the samples which they analyzed the iron content of the ash was about 0.15% with less than 10% variation from this average in the different mill fractions.

In order to determine whether our results were due to some unusual distribution of iron in the particular wheat examined or to differences in milling operations, the present studies were extended to include a number of mills operating on different wheat mixes. Table IV presents the averages obtained on the fractions from ten different mills.

TABLE IV

THE IRON CONTENT OF FRACTIONS OF WHEAT

(Dry basis)

Mill fraction	Average iron content	Iron in ash	
		Average	Range
	μg/g	%	%
Patent flour	8.4	0.180	0.125–0.239
First clear	17.4	0.219	0.165–0.250
Second clear	38.7	0.258	0.214–0.290
Red dog	96.2	0.302	0.284–0.337
Shorts	139.0	0.281	0.249–0.335
Bran	146.2	0.206	0.187–0.228
Germ	91.3	0.192	0.174–0.210
Wheat	41.6	0.232	0.198–0.262

While the actual values are not the same as those given in Table III the relative differences are similar. In each case the average percentages of iron in the ash are lowest for the patent flour and bran and highest for the low-grade and finely ground feeds. Germ is also low and wheat again occupies an intermediate position. The last column shows the range of values found. It will be noted that patent flours vary widely, since for a 0.40 ash grade the range of 0.125 to 0.239 means an iron variation of from 5 to 9.5 μg per gram.

In individual fractions, that is, those obtained from a single wheat mix, these average ratios of iron to ash will not always prevail. There is a considerable difference between different wheats and mills. In two of the series presented above the ratio was much more constant than the averages shown. One of these was a series kindly supplied by Dr. Betty Sullivan. Iron analyses were in excellent agreement between the two laboratories and the ratios of iron to ash in the patent and clears were the same as that of the wheat. Bran, and especially germ, ran somewhat lower and shorts tended to be higher, but differences were much less marked than the averages shown in the table.

Experimental

The analytical method employing carbonate fusion of the ash has already been described by the American Association of Cereal Chemists (1941). The method using acid hydrolysis was as follows:

A 2- to 5-gram sample was ashed in a porcelain crucible placed in a muffle furnace. The maximum temperature of the furnace was 575°C and total ashing time was 16 to 18 hours. After cooling, 2 ml of concentrated hydrochloric acid (cp quality) was added and the crucible, covered with a watch glass, was gently heated for a few minutes until the ash was dissipated. (Ash from whole wheat will contain carbon particles which are removed by filtration after diluting to volume. These particles contain no measurable amount of iron.) The ash solution was then transferred to a 100-ml volumetric flask and after thorough washing with distilled water was made up to volume.

Ten ml of this solution was placed in a colorimeter tube and the following solutions added: 2 ml of 2½% hydroquinone, 5 ml of acetate buffer, and 2 ml of 0.1% α,α'-dipyridyl. After thorough mixing, the color of the solution was read in an Evelyn photoelectric colorimeter.

Summary

A simple method using α,α'-dipyridyl for the determination of iron in cereals and cereal products is described. Pyrophosphate interference is eliminated from the ash either by fusion with sodium carbonate or by heating with hydrochloric acid. Losses of iron during dry ashing have not been observed. The iron analyses of wheat and its products of milling are reported.

Acknowledgment

The authors are indebted to Messrs. David Terry and John Zalar for some of the analyses reported in this paper.

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BOOK REVIEW

Chemistry of Food and Nutrition, Sixth Edition. By Henry C. Sherman. Published by the Macmillan Company, New York, N. Y. 1941. 660 pages. $3.25.

This new edition of a standard book retains many of its well known characteristics and contents but has a changed and more attractive format. Since much new information in the field of nutrition has been accumulated in the four years following the last revision, the author has had to choose topics for deletion from both old and new data in order to retain the size of the former edition. There may not be universal approval of the choices made, particularly in the treatment of the intermediary metabolism and in the elimination of most of the purely chemical discussion. Doubtless many readers of the book will applaud this trend toward the practical or applied type of treatise, even though the supply of books of the latter type is not inadequate, and there are very few indeed of the more scientific variety.

The division of space allotted to the various topics is as follows: review of the chemistry and nutritive function of carbohydrates, fats, and proteins, 12%; digestion and metabolism, 7%; energy requirement, 12%; protein requirement, 4%; minerals, 17%; vitamins, 21%; reproduction and growth, 5%; interpretation in terms of human feeding, 12%; and tables of food composition, 3%.

A generous list of references is printed at the end of each of the chapters. For most readers, however, more value might be derived from these references if they were presented as documentation of the statements in the text.

The author has adopted a philosophic and at times forensic style, particularly marked in the discussion of the calcium requirement, in presenting his interpretation of the facts of nutrition—an unusual privilege which might well be conceded in this case. The choice of data cited may not be universally accepted as the best by other workers in the field, but since choice had to be made, the author naturally selected that which was in his judgment most significant.

Certain interesting discrepancies grew out of this condition. For instance the discussion of the need for thiamin contains guarded skepticism as to the advantage of increased intake beyond the actual requirement and the general conclusion that a reasonably intelligent choice of diet will provide adequate thiamin. But much emphasis is given to the desirability of riboflavin intakes far in excess of the minimum, based chiefly upon the author's experiments with rats fed diets containing various amounts of milk.

A curious omission occurs in that there is no mention of the well known toxic effects of excess intake of vitamin D. Similarly, although the work of Jeans and Stearns upon the vitamin D requirement of infants is frequently cited, there is no reference to their experience with adverse effects of administration to infants of doses greater than 1800 U. S. units daily. This failure to include a mild warning of the danger of hypervitaminosis D is unfortunate in a book which may be for many students the sole source of knowledge of the newer nutrition.

The tables in the text as well as in the appendix are concise and complete. The table of mineral composition of foods is a unique contribution for which this book has long been esteemed and the vitamin table is an excellent compilation, expressed in terms of weight units for the most part. Another good feature of the book, important in so authoritative a volume, is the use of the proper chemical names for vitamins of known structure.

No student of nutrition should miss the pleasure and profit of reading this new edition of so important a pioneer book.

AGNES FAY MORGAN, University of California, Berkeley, California

INDEX—AUTHOR AND SUBJECT

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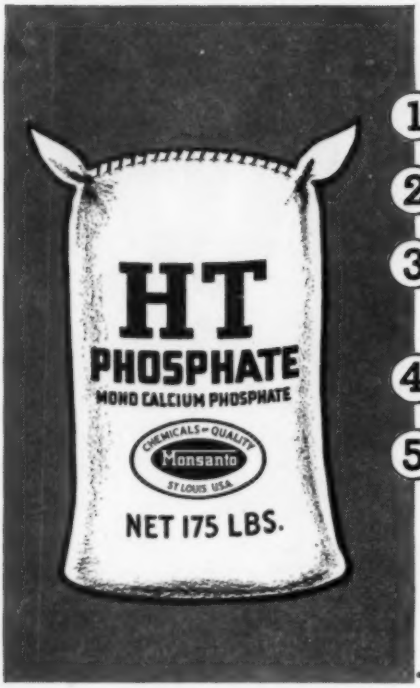

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Froedtert Grain and Malting Co., Inc., 38th and W. Grant St., Milwaukee, Wis.
Federal Mill, Inc., Lockport, N. Y.
General Mills, Inc., Chamber of Commerce, Minneapolis, Minn.
General Mills, Inc., Research Labs., 2010 E. Hennepin, Minneapolis, Minn.
Gerber Products Corp., Fremont, Michigan
Great Western Malting Co., Vancouver, Washington
Hauser, Joseph G., Dixie Portland Flour Co., 1st & Hull Sts., Richmond, Va.
Heffernan, Frank, 345 Waverly Road, Toronto, Canada
Innis, Speiden & Co., 117 Liberty St., New York City, N. Y.
Institute of American Meat Packers, 59 East Van Buren St., Chicago, Ill.
International Milling Co., Minneapolis, Minn.
Ismert Hincke Milling Co., Topeka, Kansas
Junge, Robert C., Junge Baking Co., Joplin, Mo.
Kraft Phenix Cheese Corp., 400 Rush St., Chicago, Ill.
Laboratory Construction Co., 1111 Holmes St., Kansas City, Mo.
Langendorf United Bakeries, Inc., 1160 McAllister St., San Francisco, Calif.
Library, Central Laboratories, General Foods Corp., Hoboken, New Jersey
Long, W. E., Co., 144 No. Clark St., Chicago, Ill.
Monsanto Chemical Co. 1700 S. Second St., St. Louis, Mo.
Montana Expt. Station Grain Laboratory, Bozeman, Montana
Morque, Herbert, State Mill & Elevator Co., Grand Forks, N. Dak.
National Grain Yeast Corp., 800 Mill St., Belleville, N. J.
Northwestern Miller, Minneapolis, Minn.
Novadel-Agene Corp., Newark, N. J.
P. Duff & Sons, Inc., 920–922 Duquesne Way, Pittsburgh, Pa.
Pet Milk Co., Battle Creek, Mich.
Pfaltz & Bauer, Inc., Empire State Building, New York, N. Y
Pillsbury Flour Mills Co., Minneapolis, Minn.
Quality Bakers of America, 120 West 42nd St., New York, N. Y.
Red Star Yeast & Products Co., 221 E. Buffalo St., Milwaukee, Wis.
Rumford Chemical Works, 9 Newman Avenue, Rumford, Rhode Island
Russell-Miller Milling Co., Minneapolis, Minn.
Seagram, Joseph E., & Sons, Lawrenceburg, Indiana
Siebel, E. A. & Company, 8 South Dearborn St., Chicago, Ill.
Siebel, J. E., Son's Co., 958–966 Montana St., Chicago, Ill.
Southwestern Miller, 302 Board of Trade Bldg., Kansas City, Mo.
Standard Brands Inc., 595 Madison Ave., New York, N. Y.
Standard Milling Co., Kansas City, Kansas
Standard Milling Co., 503 Seneca St., Buffalo, N. Y.
Stein-Hall Manufacturing Co., 2941 S. Ashland Ave., Chicago, Ill.
Thomas Page Milling Co., Topeka, Kansas
Victor Chemical Works, 3000 Board of Trade Bldg., Chicago, Ill.
Virginia Carolina Chemical Corp., P. O. B. 667, Richmond, Va.
Wahl-Henius Institute, Inc., 64 E. Lake St., Chicago, Ill.
Wallerstein Company, Inc., 180 Madison Ave., New York City, N. Y.
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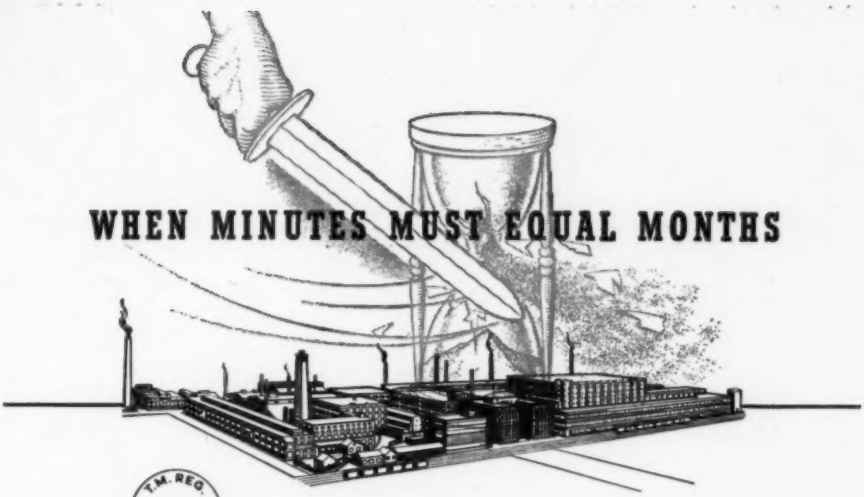

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